THE AMERICAN
JOURNAL OF PHYSIOLOGY

VOL. 36 JANUARY 1, 1915 No. 2

STUDIES ON CEREBRO-SPINAL FLUID

VIII. The Effect of Pituitary Extract Upon Its Secretion (Choroidorrhoea)

LEWIS H. WEED AND HARVEY CUSHING

From the Laboratory of Surgical Research, Harvard Medical School, Boston

Received for publication October 5, 1914

I. INTRODUCTORY

In the course of these studies we first devoted ourselves to the determination of the various pathways of escape for the fluid from the subarachnoid spaces, and it was not until some comprehension of these processes was afforded[1] that our attention was turned to the question of its elaboration. In a preceding paper it was pointed out that the fluid need not be considered as solely a product of the choroid plexuses, for evidence was presented that accretions to the fluid may be derived from the important perivascular system, which, in accordance with our conception, carries the fluid waste products of nerve cell activity toward the subarachnoid spaces.[2] The question naturally arose, do the drugs which appear to increase the flow of cerebro-spinal fluid act directly on the choroid plexuses or is their influence exerted on the cerebral capillaries in such a way that an increased amount of fluid is poured out through the perivascular lymphatics? In the attempt to answer this question it was necessary to devise some method by which the secretion from the choroid plexuses themselves could be secured apart from that possibly derived from other sources. This was at first

[1] Studies on cerebro-spinal fluid. Nos. I–VII. Jour. Med. Research, 1914. xxxi, 1 to 176.

[2] Ibid, No. IV. The dual source of cerebro-spinal fluid. Jour. Med. Research, 1914, xxxi, 109.

accomplished by the method, described in an earlier paper, of catheterizing the third ventricle through the aqueduct of Sylvius after opening the cisterna magna and exposing the fourth ventricle; and though information of value was thus secured the method is open to the many objections which apply to all extensive suboccipital procedures—the severity of the operation with its considerable trauma and loss of blood, the unavoidable immediate escape of a considerable amount of pre-formed fluid, and, in case an attempt is made to tap the cistern without the preliminary operative exposure of the occipito-atlantoid ligament, the liability of pricking the brain stem, with more or less resultant subarachnoid bleeding.

Some studies by Wegefarth[3] had demonstrated the absence of complications following a mid-line puncture of the third ventricle directly through the longitudinal sinus, and consequently we came to employ this device in our later observations (e.g. protocol of experiment LIV), for under these circumstances the operative steps preliminary to securing a uniform record of drops are greatly simplified, and as the cranial chamber remains intact there is no immediate escape of a large amount of fluid.

We have ventured to introduce the term *choroidorrhoea* as a convenient designation of a condition of increased secretory activity of the plexuses.

II. REVIEW OF LITERATURE

The early suggestion by Faivre[4] and by Luschka[5] some sixty years ago, that the choroid plexuses give rise to the fluid, was based entirely upon the presumably glandular character of these vascular structures. The first definite proof of the correctness of their assumption was not afforded until Capelletti[6] in 1900

[3] Ibid, No. VI, The Establishment of Drainage of Intra-ocular and Intracranial Fluids into the Venous System. Jour. Med. Research, 1914, xxxi, 149.

[4] Faivre, E.: Des granulationes meningiennes. Paris Thesis, 1853.

[5] Luschka, H.: Die Adergeflechte des menschlichen Gehirns, Berlin, 1855.

[6] Capelletti, L.: L'efflusso del liquido cerebro-spinale dalla fistola cefalorachidiana in condizioni normali e sotto l'influenza di alcuni farmaci. Atti dell' Accad. d. Scienze Med. e Nat. d. Ferrara, 1900, lxxiv, 85.

introduced pharmacological agents into the study of cerebro-spinal fluid production. He demonstrated a marked increase in the rate of flow of the fluid under the influence of muscarine, pilocarpine and ether, and a slowing under the influence of atropine. Two years later Pettit and Girard,[7] as reported in their exceptional monograph, correlated the conditions of increased production of the fluid with the occurrence of definite histological changes in the cells of the choroid plexuses, thus affording more convincing proof than had been presented that these structures are concerned with the elaboration of the fluid. Subsequent studies by Meek,[8] Findlay,[9] Yoshimura[10] and a number of others served to place the secretory function of the plexuses upon a still more solid basis, and Mott,[11] goes so far as to term these structures the "choroidal glands."

More recently Dixon and Halliburton[12] have presented reports dealing with the rate of production of cerebro-spinal fluid under the influence of various pharmacological agencies unemployed by Capelletti. They were apparently the first and only investigators to report upon the effects of pituitary extracts, which in their hands gave entirely negative results. They made, however, the significant observation that a marked increase in the secretion follows the intravenous injection of dried extracts of the choroid plexuses and that a slight response also occurs to extracts of brain substance itself. This demonstration of an apparent hormone action upon the production of the fluid will doubtless incite others to work in this field, and many other

[7] Pettit, A., and Girard, J.: Sur la fonction sécrétoire et la morphologie des Plexus Choroid des ventricules latéraux du Systeme nerveux central. Paris, 1902.

[8] Meek, W. J.: A study of the choroid plexus. Jour. Comp. Neurol. and Psych., 1907, xvii, 286.

[9] Findlay, J. W.. The choroid plexuses of the lateral ventricles of the brain: their histology normal and pathological. Brain, 1899, xxii, 161.

[10] Yoshimura, K.: Die histochemische Verhalten des menschlichen Plexus choroideus. Arbeiten a. d. neurol. Inst. a. d. Wien Univ., 1909, xviii, 1.

[11] Mott, F. W.: "The Oliver-Sharpey lectures on cerebro-spinal fluid." Lancet, 1910, ii, 1.

[12] Dixon, W. E., and Halliburton, W. D.: The action of the choroid plexuses on the secretion of cerebro-spinal fluid. Jour. Physiol., 1910, xl, 30; Cerebro-spinal fluid, I. The secretion of the fluid. Ibid. 1913, lxvii, 215.

tissue extracts will undoubtedly be tested.[13] Most of our own experiments were made before the publication of Dixon and Halliburton's complete paper, and our experiences with ether, adrenaline, pilocarpine and other substances are more or less in accord with those of our predecessors. In this report we shall dwell exclusively upon the effects observed with pituitary extracts.

III. METHODS EMPLOYED

The rate of production of cerebro-spinal fluid has been studied by previous observers in various ways. Cavazzani[14] established a cerebro-spinal fistula for the purpose, but in the majority of cases hollow needles or cannulae have been introduced into the great subarachnoid cistern beneath the occipito-atlantoid ligament or elsewhere into the subarachnoid spaces of the spinal canal.

The results obtained by these customary methods, as has been indicated, are susceptible to misinterpretations, and in the attempt to avoid them, in our early studies use was made of ventricular catheterization through the iter. The small paraffined silk catheters employed were calibrated in terms of millimeters of water (i.e. the height of a column of water sufficient to cause fluid to drop from the end); and after operative exposure of the fourth ventricle practise makes it possible to safely introduce one of them along its floor and through the aqueduct of Sylvius into the third ventricle. The resistance of the catheters was such that pressures approximating the normal were maintained within the ventricles. In this way the normal intraventricular pressure conditions were supposedly not interfered with, and the results obtained promised a greater degree of reliability than when, under the usual conditions of such an experiment, the intraventricular tension is allowed to fall almost to zero.

[13] Frazier reports briefly upon experiences in this direction and states that thyroid preparations appear to inhibit the secretion. (The cerebro-spinal fluid as a problem in intracranial surgery. Jour. Amer. Med. Assn., 1914, lxiii, 287.)

[14] Cavazzani, E.: La fistola cefalorachidiana. Atti dell' Accad. d. Scienze Med. e Nat. di Ferrara, 1899, lxxiii, 27.

The chief disadvantage of this method lay in the severity of the preliminary operative procedure, and indeed the mere free exposure of the occipito-atlantoid ligament in the canine with the subsequent introduction of a needle into the posterior cistern, after the manner usually employed, is open to the same objection. To avoid the loss of blood and shock incidental to this extensive exposure Dixon and Halliburton inserted the needle through the soft parts and ligament into the cistern after making little more than a skin incision; but there is always danger under these circumstances of pricking the nervous axis and of causing subarachnoid bleeding. Even with a successful puncture of the cistern by this method and the use of a calibrated needle we felt, for the reasons given, that our observations would be more dependable could our records be made from the intraventricular rather than from the subarachnoid fluid spaces.

We finally hit upon the far simpler method of introducing the calibrated needle into the third ventricle by a callosal puncture—a method which, besides its simplicity, has the additional advantage of tapping the ventricle, with the skull and meninges left intact, so that the intracranial physical conditions remain unaltered and there is no primary gush of fluid such as occurs when the cistern is entered through the occipito-atlantoid ligament.

In this procedure a mid-line perforation through the bone and down to the longitudinal sinus is made with a small drill the size of the calibrated trocar which is to follow it, so that the latter when introduced stays securely in place. This small opening is occluded with wax or plastacene and the trocar and cannula are then introduced directly through the sinus and alongside the falx into the third ventricle—a depth of 2.5 or 3.5 cm., depending on the size of the animal (fig. 1). The trocar is then withdrawn from the cannula and clear fluid, without the usual primary outrush, begins to drop at regular intervals which can be recorded.

In all such observations the anaesthetic requires particular attention. As both chloroform and ether are known to affect the flow of cerebro-spinal fluid, they must be administered more

evenly than is possible when either of these volatile anaesthetics is given in a cone or by the open drop method. Only by the employment of intratracheal insufflation with an unchanging tension of the anaesthetic can a long enduring, constant level of blood pressure be insured during the number of hours necessary for the experiment.[15]

The experiments were conducted with the subject (dog or cat) in the ventral position. The arterial blood pressure as a rule was recorded from the carotid, for though the femoral was

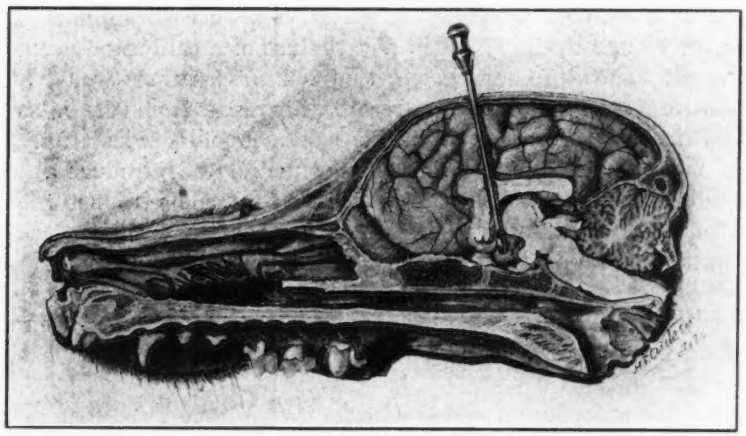


Fig. 1. Sagittal Section of Canine Skull, Showing Point of Election for Tapping Intraventricular Fluid.

occasionally used, the ligation of one carotid apparently has no effect on the secretion of the fluid. The respiration tambour recorded inspiration by an upward stroke. In certain observations the urinary outflow was also included on the record, the drops of urine, issuing from a catheter inserted in a ureter extraperitoneally in the loin, being indicated by an appropriate signal.

[15] Dixon and Halliburton attempted to overcome these difficulties by using morphine and urethane as an anaesthetic. We have had less success with this method.

All injections were made intravenously, usually into the small saphenous vein, readily exposed on the posterior aspect of the dog's leg and by far the most satisfactory vessel in which to make repeated injections with an animal in this position.

IV. THE EFFECT OF PITUITARY EXTRACTS UPON THE FLUID PRODUCTION

It is well known that intravenous injections of suitable amounts of posterior lobe preparations cause a rather prolonged rise in arterial pressure, usually preceded by an initial fall of variable duration. This typical haemodynamic response, first noted by Schäfer,[16] was attributed by Howell[17] to substances confined to the pars nervosa. Repeated injections of these extracts at short intervals tend in the main to increase the depressor phase at the expense of the pressor.

Experience has taught us that there is great variability in these haemodynamic responses to posterior lobe extracts, whether due to the method of preparation, to the state (possibly seasonal) of inactivity or otherwise of the gland from which the extracts are prepared, or to the individual susceptibility to the extracts (possibly again seasonal or periodic) on the part of the animals on which they are tested. Undoubtedly many of the contradictory results of the pharmacological studies on hypophysial extracts are attributable to these several sources of confusion. Moreover there are in all likelihood several active principles or hormones, rather than a single one, contained in the gland, and, by the particular method of preparation employed, one or another of the substances may fail to be extracted even should it actually have been present in significant amounts in the fresh glands. Thus one may secure preparations in which either the presser or the depressor response is paramount, or preparations in which there is no haemodynamic response whatsoever, and yet the same preparations may cause marked diuresis, or glycosuria,

[16] Schäfer, E. A.: The physiological effects of extracts of the pituitary body. Jour. Physiol., 1899–1900, xxv, 87.

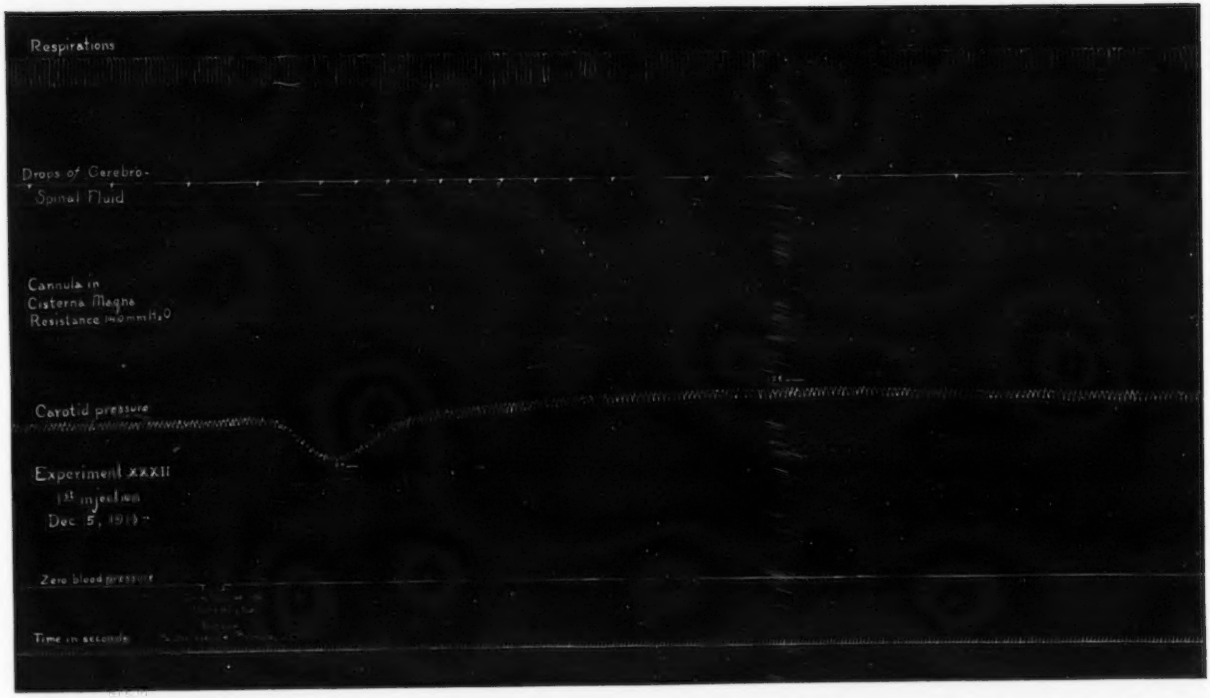
[17] Howell, W. H.: The physiological effects of extracts of the hypophysis cerebri and infundibular body. Jour. Exper. Med., 1898, iii, 245.

or powerfully contract smooth muscle, and so on. We have observed, too, that successive proprietary samples extracted presumably by the same technical method are most variable in their potency, so that until the pharmacologist can actually isolate, and perhaps synthesize, the substance or substances in question, making it possible to estimate accurately the dosage, negative reactions with the substances at hand are far less significant than positive ones.

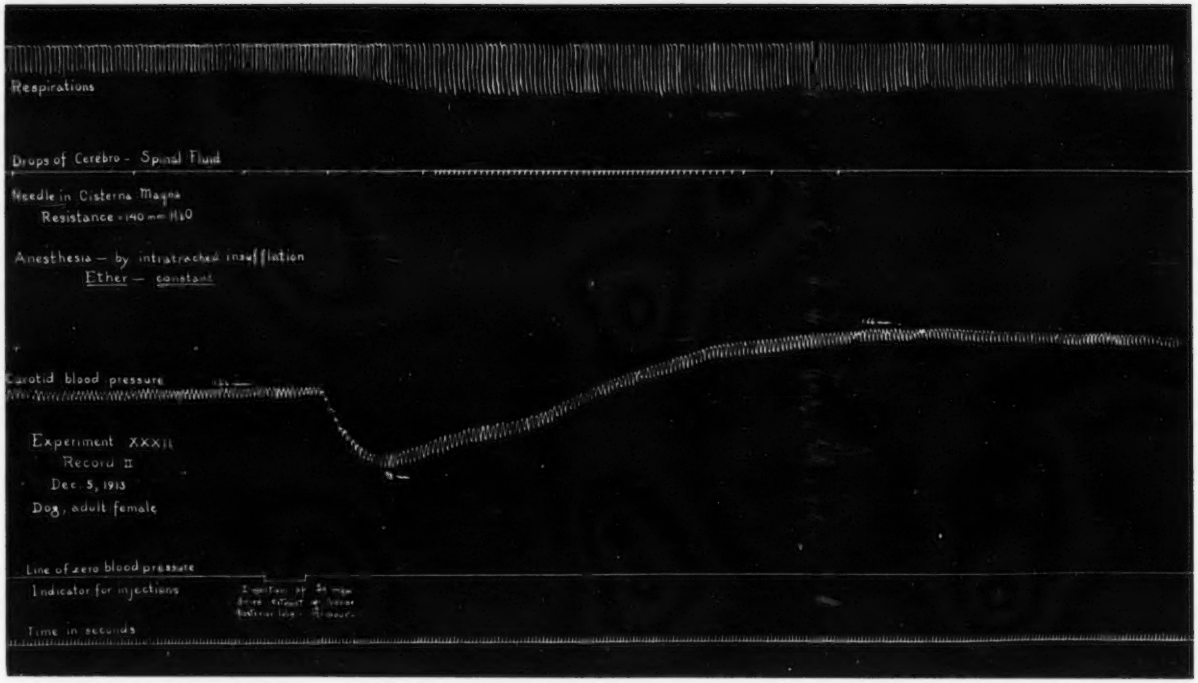
As it is true of the haemodynamic or diuretic responses, so in the case of the cerebro-spinal fluid production great variation has been observed with the different extracts which have been used. The most active preparations in our experience have been the dried extracts of fresh bovine posterior lobes prepared in this laboratory and also some powdered extracts dried *in vacuo* for us by Armour and Company. We have compared these dried extracts with the various fluid preparations of the different pharmaceutical firms (Parke, Davis and Company's "Pituitrin;" Lucius, Meister, and Brünig's "Hypophysin;" Armour and Company's "Pituitary Liquid," and the "Pituitary Extract" of Schering and Glatz), and though none of them has been as potent as the dried extracts, nevertheless an augmentation of the flow of cerebro-spinal fluid has been obtained at one time or another from each of these fluid preparations.

A fairly typical though slight response to the injection of 1 cc. of "Hypophysin" is shown in Record I, the fluid in this experiment (No. XXXII) issuing from a calibrated needle in the cisterna magna with a regular outflow established at the rate of a drop about every twenty seconds. Following the injections the characteristic though slight vasomotor response (with a primary depressor phase from 114 mm. to 84 mm. and a subsequent rise to 128 mm. Hg.) occurred, with a moderate acceleration of fluid outflow at the rate of a drop every eight seconds for the succeeding two minutes, followed by a subsequent slowing of the pre-injection rate.

Subsequently (cf. Record II) in the course of this same experiment (No. XXXII), after the re-establishment of a regular fluid outflow, at the rate of a drop every thirty seconds, an injection



Record I. Experiment XXXII. December 5, 1913. Dog. Intratracheal anaesthesia: cannula in cisterna magna: slight increase in flow after injection of 1 cc. "Hypophysin," with slight blood pressure response.



Record II. Conditions of experiment as in Record I: marked temporary choroidorrhoea with more pronounced vasomotor response, following the injection of 50 mgm. of dried bovine posterior lobe extract.

of 50 mgm. of the dried bovine extract in 3 cc. of salt solution gave a more pronounced response, with the typical fall (from 126 to 78 mm. Hg.) and subsequent rise (to 166 mm.) in blood pressure, the former phase being somewhat more prolonged than usual. As will be seen, the onset of the sudden outpouring of cerebro-spinal fluid corresponded with the period of lowest blood pressure and ceased at the crest of the pressor wave a minute and a half later, following the escape of 60 drops of fluid, after which there was an abrupt cessation in the flow. Coincident with this period of supposed choroidorrhoea a slight amplitude of the respiratory stroke is registered,[18] and without further data one might be inclined to attribute the response to the physical expulsion of pre-formed fluid brought about by an increase of intracranial tension from asphyxia or some other cause. On this basis, however, one would not have expected the fluid to be expelled during the depressor, but rather through the pressor, phase, as is the case after compression of the jugulars, when there is apt to be a temporary flow from the cannula, due undoubtedly to mechanical rather than secretory influences.

It will be seen from the succeeding two records, taken from other animals in which the characteristic initial depressor phase in the haemodynamic responses did not occur, that very similar responses on the part of the cerebro-spinal fluid were nevertheless elicited.

In Record III, from a canine experiment (No. LI) the fluid, at the time of the injection of 100 mgm. of dried bovine extract, was issuing from the cistern catheter at the rate of 8 drops a minute, and immediately upon the injection the rate quickened to 50 drops for the following minute, this response being inaugurated before the initial stage of the haemodynamic pressor

[18] Dixon and Halliburton (*loc. cit.*) state that an increase in the rate or amplitude of respiration facilitates the normal processes of escape of the fluid with consequent decrease in the flow from the cannula. Here the marked increase in fluid outflow is synchronous with the increase in the respiratory movements. These suction effects of respiration do not appear to us to have influenced the responses in any of the experiments, in most of which the normal pathways of absorption, via the meninges, were excluded by the methods employed, of intraventricular insertion of the catheter or cannula.

change set in. There was a subsequent gradual retardation of the flow, with temporary complete cessation after seven minutes. The respirations were not recorded in this experiment but here again, as in Record II, the period of increased flow coincided more or less sharply with the period of rising blood pressure. The following record, however, shows that the choroidorrhoea may occur with a persisting level of blood pressure.

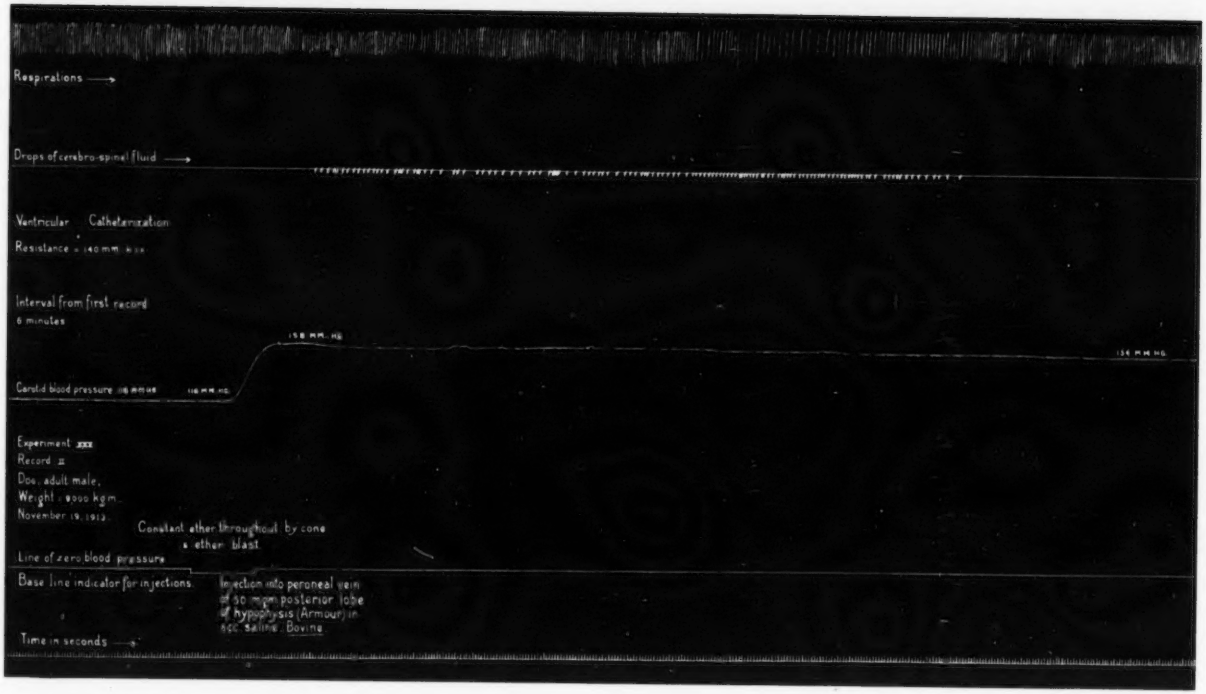
In Record IV, from a dog with a calibrated catheter in the aqueduct, (Experiment No. XXV) is shown an extreme temporary choroidorrhoea. The animal had already received one injection of an extract which after a slight response had been followed by a period of cessation in the flow. On this second occasion 50 mgm. of dried bovine posterior lobe extract was given, causing an enduring primary pressor response (from 116 to 158 mm. Hg.) with an immediate outpouring in the course of the next 4 minutes of 128 drops (about 8 cc.) of fluid—far more than the canine ventricles normally contain. This was followed in turn by a second prompt cessation without any apparent change, as will be noted, either in blood pressure or respiration.

In order to emphasize still further the lack of dependence of these fluid responses upon haemodynamic or other obvious mechanical changes in intracranial tension the following protocol may be given in full.

Experiment LIV. July 17, 1914. Adult female 10.5 kilo dog. *9.15 a.m.* Intratracheal anaesthesia: insertion of catheter in right ureter, exposed extraperitoneally by posterior incision in flank: introduction of blood pressure cannula in right carotid: exposure of posterior saphenous vein in left leg: ventriculo-callosal puncture: attachment of carotid cannula to manometer and kymographion. Blood pressure 136 mm. Hg., practically uninfluenced by respiration, which is panting, about 80 to the minute (and which continued so during the experiment). Cerebro-spinal fluid gradually slowing from initial rate of 22 drops per minute to about 10 per minute at time of first injection. Urine flowing at rate of 3 drops per minute from the single ureter: no sugar in first specimen.

10.17 a.m. First injection (cf. *Record V*): 4 cc. of a concentrated 20 to 1 solution of bovine cerebro-spinal fluid, followed by a marked

Record III. Experiment LI. April 6, 1914. Dog. Intratracheal anaesthesia: calibrated catheter in cisterna magna: acceleration of flow with pressor response and no initial depressor phase.



Record IV. Experiment XXV. November 19, 1913. Dog. Catheterization through aqueduct: marked temporary choroidorrhoea associated with long-enduring and stationary pressor response.

depressor response (from 136 to 64 mm. Hg.) and slight subsequent and prolonged rise (to 150 mm. Hg.) without apparent influence on the rate of cerebro-spinal fluid flow. An immediate temporary check, however, occurred in the outflow of urine.

10.25 a.m. Second injection (cf. *Record VI*): 100 mgm. of posterior lobe powder (Armour) dried *in vacuo* at 38°. Prompt cerebro-spinal fluid response lasting 3 minutes, with increase in drops from 4 per minute to 13 per minute (over 2 cc. in all), coincident with an extreme depressor response. This was followed by an abrupt and prolonged rise in blood pressure (to 176 and finally 192) with a cessation in cerebro-spinal flow, which endured for about thirty minutes, when (at *10.49 a.m.*) there was a slow return of drops, at the rate of one per minute.

Seven minutes after the injection (i.e. at *10.32 a.m.*) an abrupt polyuria started up and continued for the following half hour, during which time there was such a rapid flow that 27 cc. were collected from the single ureter, and probably double that amount was secreted from the two kidneys. The urine contained a high percentage of sugar.

11.12 a.m. After a long-enduring pressor response the carotid registration is again at 150. Polyuria continues. A cerebro-spinal fluid drop every 2 minutes. *Third injection:* 4 cc. of "Hypophysin," causing a slight vasomotor response (150 to 116 to 172 mm. Hg.) and coincident cessation of cerebro-spinal fluid for the following 13 minutes, after which time (at 11.25) fluid again began to appear, and continued at the rate of about 3 drops per minute for the interval between 11.30 a.m. and 12.40 p.m., during which time the drum was stopped and a new record prepared.

12.50 p.m. Carotid pressure at constant level of 148 mm. Cerebro-spinal fluid drops regular, 3 per minute. *Fourth injection:* 100 mgm. of dried posterior lobe extract (laboratory preparation) followed by marked vasomotor response with a double depressor phase (148 to 120: 158 to 126 mm.) and subsequent prolonged rise (at 162 mm.): also by a temporary acceleration of cerebro-spinal fluid outflow and subsequent slowing to about one drop per minute; also by a temporary cessation of urine for 4 minutes and then a sharp polyuria at the rate of 9 drops per minute.

1.05 p.m. Fifth injection: 5 cc. of the Schering preparation followed by a very slight vascular response (132 to 120 to 138 mm.) with a slight acceleration of the cerebro-spinal outflow but no effect on the steady flow of urine.

1.17 p.m. Sixth injection. Repetition of 100 mgm. Armour preparation dried *in vacuo*, followed by an immediate quickening of cerebro-spinal fluid drops from 1 to 6 per minute and a temporary 3 minute cessation of urine in association with a marked vasomotor response, (with primary fall from 142 mm. to 114 mm. and subsequent prolonged rise, finally reaching 196 mm. Hg.).

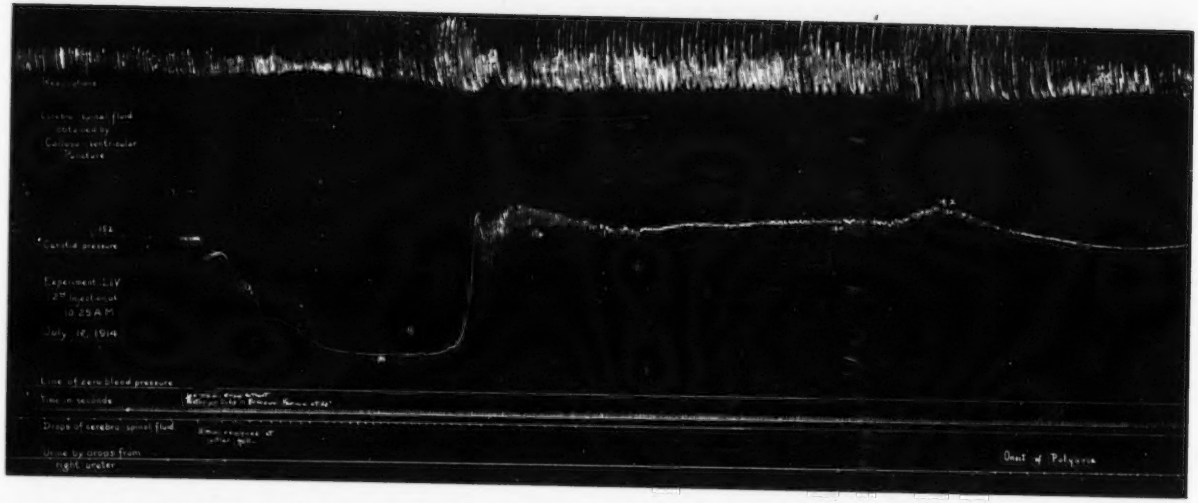
1.30 p.m. Seventh injection. Repetition of the above with a similar good response associated with definite choroidorrhoea (7 drops per minute), followed by practical cessation.

1.35 p.m. Eighth injection: 3 cc. of ether followed by a sharp fall in blood pressure from 156 to 64 mm. Hg., accompanied by the appearance of a few drops of cerebro-spinal fluid.

1.40 p.m. Ninth injection: 5 cc. of ether to produce fatality, followed by a prompt fall of blood pressure to zero, and respiratory cessation, associated with an immediate choroidorrhoea (14 drops in the first minute) which continued for twenty-one minutes after death: 4.1 cc. of cerebro-spinal fluid secured in all. The urine, which up to this time had been dropping rapidly, ceased abruptly, coincident with the fall of the blood pressure to zero.

This protocol emphasizes a number of things: the variability of the responses in the same animal to different extracts: the fact that it is the substance injected which causes the increased fluid outflow rather than the variations in blood pressure associated with possible changes in the physical conditions within the skull (cf. Records V and VI): the fact that even in a prolonged experiment (in this case nearly four hours) the secretory activity need not be exhausted by repeated injections, provided that an extreme choroidorrhoea has not been provoked at any one time: and further that even when the responses to pituitary extracts have weakened, an injection of ether may arouse an active choroidorrhoea. That the pituitary substances, particularly the dried extracts, were active in other directions than on the cardio-vascular system is shown by the glycosuria following the first injection, as well as by the diuretic responses, and it may be noted that the latter effect after each potent injection was usually preceded by a latent period of two or three minutes, whereas the choroid effect was immediate and often briefly anticipated the characteristic haemodynamic response.

Record V. Experiment LIV. July 17, 1914. Dog. Intratracheal anaesthesia: callosal puncture. Showing marked depressor response (to first injection of 4 cc. bovine cerebro-spinal fluid in 20 to 1 concentration), without effect on cerebro-spinal outflow.



Record VI. Experiment LIV (second injection). Showing immediate temporary choroidorrhoea associated with depressor response after injection of 100 mgm. posterior lobe extract (Armour) dried *in vacuo*. Polyuria (with glycosuria) after latent period of seven minutes.

On a number of occasions we have observed an increase in the cerebro-spinal fluid outflow in association with equally extreme initial depressor responses on the part of the cardio-vascular system, but a comparison of the effects of the consecutive first and second injections (Records V and VI) of the foregoing experiment makes it seem improbable that pre-formed fluid could merely have been extruded under these conditions by intracranial venous stasis through asphyxia, for were this the case, on the restoration of free circulation one would have expected a recession of the fluid into the cannula. Such a recession, as will be pointed out later on, normally follows temporary increases in the outflow which are obviously due to mechanical causes, as for example after compression of the jugulars: and for this same reason we are inclined to attribute to physical influences the temporary outflow which accompanies the marked rise in blood pressure after injections of adrenaline.

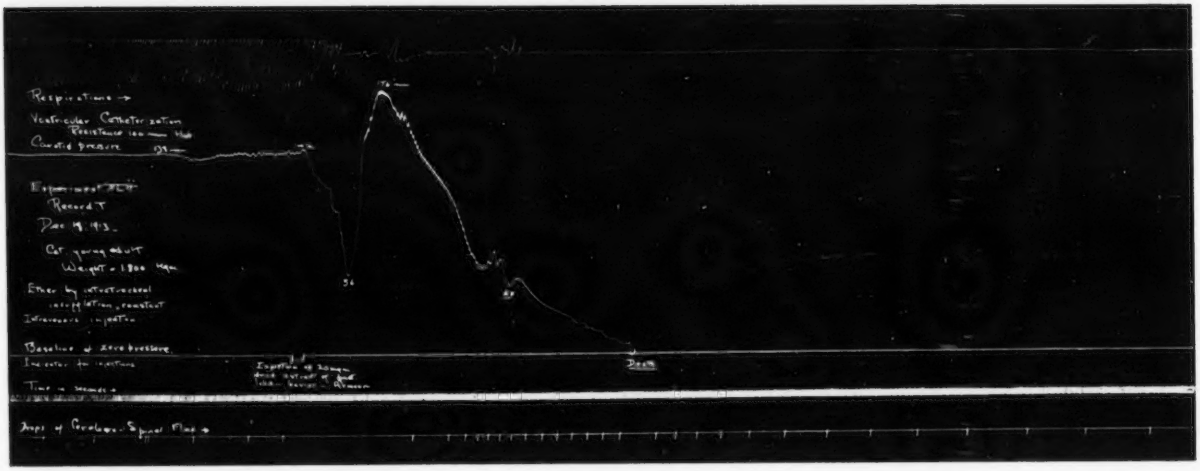
Certain of the smaller animals, as cats and rabbits, at times appear to be extremely susceptible to the haemodynamic effects of pituitary extracts, and prolonged depressor responses may occur and prove fatal, or on the other hand the cardio-vascular readjustment may be abrupt (as in Record VI), with the exchange from the low to a high pressor phase taking place in a few seconds. An illustration of a prolonged depressor phase with recovery is shown in Record VII, giving the response in a cat to the injection of 1.0 cc. of Armour's "Pituitary Liquid." In this experiment the fluid, secured by ventricular catheterization, was dropping before the injection at the rate of a drop every two minutes, and during the $3\frac{1}{2}$ minutes of the extreme depressor phase (with a blood pressure almost at zero, the pulse pressure almost nil, and nearly total respiratory cessation) the flow increased to a drop every thirty seconds. This response might possibly be attributed to asphyxiation, for the flow practically ceased after the onset of the pressor phase with its accompanying increased amplitude of respiration. However, the same response may occur (cf. Record VI) when the respiratory amplitude is increased during rather than after a profound depressor phase.

We are at a loss to account for the extraordinary phenomenon of a prolonged post-mortem outflow of fluid. This was mentioned in the protocol given above as taking place after a fatality from ether, and we have seen a number of similar responses after fatalities provoked by the injection of toxic doses of ether or of pituitary extracts, with a continued flow, which in some cases has endured for an hour after death.

An example is given in Record VIII, from a cat with ventricular catheterization. After the injection of 20 mgm. of dried extract there occurred an exaggeration of the usual vasomotor responses—a sharp fall, accompanied by respiratory cessation, then an equally sharp rise in pressure, followed in turn by a second precipitous fall to zero, with fatality. The fluid had been dropping from the catheter at the rate approximately of one drop a minute. There was a temporary cessation for 2 minutes after the injection and then, coincident with the final fall in pressure, the fluid began to flow rapidly at the rate of 4 drops a minute, until the death of the animal four minutes later, after which, gradually slowing, it continued to drop for an hour after the cessation of the circulation, 3 cc. being collected in all. Only the first 12 minutes of this remarkable response is shown in the reproduced fragment of the record.

Ordinarily after the death of an animal the fluid promptly recedes in the cannula, so that under these circumstances of a prolonged post-mortem flow some condition must serve either to expel the remaining pre-formed fluid (and 3 cc. is an abnormally large content for the ventricles of a cat) or else the fluid must continue to be produced for some time after the cessation of circulation. The response might possibly be attributed to asphyxiation, for the respiration ceased (cf. Record VIII) before the heart beat, or to an increase in tension from a post-mortem oedema of the brain. It is difficult to believe that the plexus could continue to secrete after the complete cessation of circulation, no matter what the ante-mortem stimulus might have been.

Record VII. Experiment XLI (second injection). December 18, 1913. Cat. Intratracheal anaesthesia: catheterization through aqueduct: injection of 1 cc. "Pituitary Liquid" (Armour). Showing marked depressor phase with temporary increase in fluid outflow.



Record VIII. Experiment XLII (first and fatal injection). December 19, 1913. Cat. Intratracheal anaesthesia: catheterization through aqueduct: injection of 20 mgm. dried posterior lobe extract (Armour). Showing extreme vasomotor response with fatality and prolonged post-mortem choroidorrhoea.

V. IS THE APPARENT RESPONSE MECHANICAL OR SECRETORY?

Insufficient attention, it seems to us, has been paid to the physical conditions which underlie the production and expulsion of the fluid through the experimental cannulae. Dixon and Halliburton[19] alone appear to have subjected their results to proper standards for differentiation of mechanical and secretory effects.

The difference in tension between the capillary pressure in the choroid plexuses and that of the surrounding fluid may be termed 'the secretory pressure.' In the apparent absence of a cerebral vasomotor mechanism, or, at all events, in the absence of one which acts in conjunction with the system distributed to the body as a whole, any constriction of the splanchnic or peripheral field is followed by a cerebral hyperaemia and, according to the generally accepted view, by an increase in the intracranial tension. But within the closed cranial chamber any change in vascularity is immediately communicated to the fluid and hence as long as the chamber is intact there need necessarily be no alteration of the secretory pressure, although some of the preformed fluid, under these circumstances, is doubtless forced out through the normal channels of outflow. Hence on theoretical grounds it seems improbable that an increase in vascular pressures will cause an increased production of cerebro-spinal fluid, provided the closed system is maintained.

Quite dissimilar from this normal state are the intracranial conditions in any experiment in which a hollow tube has been introduced into some part of the fluid pathway by any method which permits decompression effects to be felt with lowering of the intracranial tension. Here the capillary resistance of the introduced cannula determines the resulting cerebro-spinal fluid pressure. The arachnoid or intraventricular tension must remain constant or at least never exceed that of the resistance established by the cannula. A rise, then, in blood pressure with a corresponding increase in capillary tension will necessarily increase the secretory pressure. Whether under these conditions

[19] Loc. Cit. Jour. Physiol. 1913, xlvii, 215.

the choroid plexus will become more permeable to the passage of fluid and thus the amount of cerebro-spinal fluid be increased is an undetermined question which presents great technical difficulties of solution. It is the generally accepted view that a probable increase of fluid production does occur under these circumstances, but the arguments are not entirely conclusive.

More important for our present considerations is the mechanical extrusion of the cerebro-spinal fluid under the influence of changes in intracranial vascularity. A cerebral hyperaemia brought about by any agency which increases blood pressure necessarily constricts the space properly filled by fluid which, under these circumstances, in normal conditions must escape by its natural outlets connected with the subarachnoid spaces, or under the experimental conditions must drop with increased frequency from the end of the cannula which leads from the ventricles.

Venous pressures likewise which are particularly under the influence of respiratory conditions unquestionably may exert considerable effect upon the secretory pressure through alterations in the capillary tension, for as Starling has demonstrated, capillary pressure follows more closely upon alterations in venous pressure than upon far greater changes in the arterial tonus. Hence, under the conditions of our experiments a marked fall in arterial pressure may be accompanied by an equivalent rise in venous tension so that the secretory pressure may remain unchanged or even be increased. Venous congestion therefore may also serve to decrease the potential fluid space, with resultant extrusion of fluid until balance be again restored. In view therefore of the possible influence of these physical factors on the discharge of cerebro-spinal fluid, it is hazardous to assume that certain agents stimulate the secretion of the choroid plexuses, merely because of an observed increase in flow from a cannula.

Were it possible to measure the cerebral capillary pressure many of these difficulties of distinguishing the agents which act mechanically from those which truly excite secretion might be solved. A convenient though less exact method of differentiation between the two processes is afforded by the employment of catheters or of cannulae which are sufficiently transparent to

allow the pulsating level of the fluid to be observed through their walls. In the course of an experiment, with the fluid dropping regularly from such a catheter, if a purely mechanical and temporary means of increasing the cerebral arterial or venous vascularity be employed, there will occur a definite and marked acceleration in the rate of flow, and very quickly the production of fluid will become constant, dependent on the new secretory balance. Then with the subsequent lowering of the congestion on the arterial or venous side as the case may be, the fluid will be observed to recede into the cannula, to compensate for the decreasing vascularity. On the other hand, if fluid is actually being secreted no such recession is observed, and though the rate may be slowed fluid continues to appear in spite of the relatively devascularized brain. This simple expedient, which Dixon and Halliburton have commented upon, of observing the recession of fluid into the cannula is oftentimes an aid in distinguishing the mechanical from the truly excitatory effects.

Another convenient check lies in the important fact that in most of these experiments the recorded drops of fluid issue from a catheter in the ventricle rather than from one inserted in a subarachnoid cistern, for as we have shown in an earlier paper, the arachnoidal villi constitute the normal channels of escape and the outflow of fluid from the subarachnoid spaces is therefore likely to be more subject to fluctuations than the outflow from the ventricles. It is quite possible, for example, that the prolonged post-mortem flow from the ventricles which we have so often observed may be less likely to occur from a cannula in the subarachnoid spaces; for ordinarily, with cessation of the circulation and resultant cerebral anaemia, the fluid immediately recedes into the catheter, being drawn upon doubtless to fill the perivascular spaces of the more or less devascularized brain. The dilatation of these spaces in the brains of animals in which arterial ligations have produced cerebral anaemia is a phenomenon which Mott was the first to point out. If these post-mortem responses are truly excitatory rather than mechanical the increased secretion apparently suffices to overçome the call for fluid on the part of the anaemic brain and to maintain the long-

continued flow of fluid, which in many cases has certainly been more than the normal ventricles could possibly have held.

Another factor which suggests that the posterior lobe preparations serve to excite secretion is the apparent fatiguability of the response on repeated injections, even though practically the same vasomotor conditions prevail. With time given for the reaccumulation of fluid one would expect a repetition of the cerebro-spinal fluid response invariably to accompany a repetition of the haemodynamic and respiratory reaction were the effect purely mechanical. We have shown that an extreme augmentation in the rate of fluid outflow is usually followed by an abrupt and persisting cessation of the flow[20] (e.g. Records II and IV): in other instances that a cessation of the flow occurs but is more gradual in onset (e.g. Record III): in still others the flow though slowed may continue after its temporary augmentation (e.g. Record I). In some animals after a prolonged cessation the flow may be incited by a second and even by several subsequent injections (e.g. Protocol of Experiment LIV), but after one outspoken reaction a subsequent response to pituitary preparations is apt to be much less striking even though an abundant flow may be obtained by the injection of other substances, ether seeming to be especially potent in this respect. It would appear that the fatigue threshold of the choroid plexuses is much lower to pituitary extracts than is that of the vasomotor system for the same substances. It is possible too that the choroid cells may in some cases respond to smaller doses than those required for the typical vasomotor reaction, for in a few instances in which the amount of the extract has failed to provoke any apparent change in the blood pressure a brief but definite increase in the rate of the outflow of cerebro-spinal fluid has occurred.

[20] It may again be emphasized in this connection that in the case of a permanent cessation of the flow after an outspoken primary response subsequent injections of an active preparation, which calls out anew a pronounced blood pressure response, may serve to express a few drops of fluid from the cannula followed by an immediate recession of the fluid. This we regard as purely a mechanical effect.

VI. RECAPITULATION AND CONCLUSION

Extracts of the posterior lobe of the hypophysis introduced intravenously[21] serve to discharge cerebro-spinal fluid from a calibrated catheter introduced in its pathway (ventricle or subarachnoid cistern). Positive responses occur to the injection of nearly all the fluid proprietary preparations, though in our hands the desiccated extracts have proved to be the more active. The possible increment to the fluid through increased permeability of the cerebral capillaries and drainage out along the perivascular lymphatics is excluded in most of these experiments by the method of tapping the fluid pathway in the third ventricle rather than in the cisterna magna. The response is seen to occur under most variable conditions and appears to be independent of respiratory influences or haemodynamic reactions, for it may coincide with periods of respiratory cessation or acceleration or with periods of arterial hypertension or depression. A prolonged flow from the ventricle may continue even after death and may reach an amount apparently in excess of the normal content of the ventricles. That there is an actual increase in the amount of fluid secretion is shown by its non-recession into the cannula following periods of increased cerebral vascularity as well as by the measurement of the amounts discharged. The evidence inclines us to believe that the outflow under the circumstances of our experiments represents an actual secretory response rather than an expulsion of pre-formed fluid due to physical conditions resulting in changes in the volume of the brain.

Hence the conclusion may be justified that:

Extracts of the posterior lobe of the hypophysis increase the rate of production of cerebro-spinal fluid (choroidorrhoea) by stimulating the secretory activity of the choroid plexuses.

[21] In a few experiments we have attempted, without success, to excite choroid secretion by stimulating the superior cervical ganglion, in the expectation of discharging the hypophysial secretion in this way (cf. Weed, Cushing and Jacobson: The Autonomic Control of the Pituitary Body. Bull. Johns Hopkins Hosp., 1913, xxiv, 40-52) and also by injecting extracts into the ventricle itself on the view that the presence of posterior lobe secretion in the fluid itself as well as in the circulation may excite secretion (cf. Cushing and Goetsch: The Secretion of the Posterior Lobe of the Pituitary Body and its Presence in the Cerebro-spinal Fluid. Amer. Jour. Physiol., 1910, xxvii, 60-86.)

THE BLOOD PRESSURE DURING VOMITING

CLYDE BROOKS AND ARNO B. LUCKHARDT

From the Laboratory of Physiology and Pharmacology, University of Pittsburgh, and the Hull Laboratory of Physiology, University of Chicago

Received for publication October 8, 1914

The present work is a continuation of the studies of the blood pressure and its variations in the unanesthetized animal under different normal and pathological conditions. The methods adopted for taking the blood pressure are those previously described.[1]

The object of the present research was to study the blood pressure preceding, during, and following the act of vomiting; and to ascertain as far as possible the mechanism of the changes in the blood pressure or the regulation and control of the blood pressure at such a time.

Before this work was begun, we assumed that during vomiting the blood pressure was greatly heightened. This assumption appears to be generally prevalent. Text-books as a rule do not mention blood pressure changes during vomiting. In those text-books in which it is mentioned, there is no statement of facts or experimental work done; but merely indirect statements inferring that the blood pressure is greatly augmented during vomiting.

Clinical literature contains a good many indirect references to this subject and physicians are warned not to give emetics to patients whose arteries are atheromatous lest there be a rupture of the blood vessels.

The first tracing taken by us was during a study on the unanesthetized dog under the action of digitalis (with Dr. H. G.

A preliminary report of this work was made before the American Physiological Society at Cleveland, December, 1912.

[1] Brooks, C., Heart, 1910, vol. 2, p. 5.

Schleiter). It showed that the blood pressure, instead of rising during vomiting fell rather sharply to a low point and after the act of vomiting, rose rapidly to the normal or slightly above the normal. This was followed by a series of experiments carried on in the Hull Laboratory of Physiology, University of Chicago and later other experiments at the Laboratories of Physiology and Pharmacology at the University of Pittsburgh.

Six months after our paper had been presented at Cleveland[2] while attending the Minneapolis meeting of the American Medical Association, June, 1913, rumors were heard of older literature on the subject of the blood pressure during vomiting. Dr. Cannon referred us to Dr. Gatch, who he said, had found an important reference to this subject. Dr. Gatch himself stated that his idea was obtained from a reference in Hall's Text Book of Physiology. Dr. Hall writes, in part: "Traube was the first to show that at the commencement of vomiting typical vagus pulsations occur, resulting in a lowering of the general arterial pressure. At the termination of the attack the pulse becomes accelerated and the arterial pressure rises even above the normal."[3]

About this time (June, 1913) an article by Hesse[4] directed our attention to a remarkable volume by Guinard[5] on the subject of morphine and apomorphine in which we found descriptions of experiments and tracings the results of which are in complete accord with those reported in our preliminary report.

[2] An abstract of the paper prepared for publication in the Proceedings of the American Physiological Society of that year (1912) was refused publication because of its length. A shorter abstract reached the editor of the American Journal of Physiology too late for publication.

[3] A Text Book of Physiology, Winfield S. Hall, Lea Brothers & Co., 1905, pp. 411–412. The reference to Traube's work given at the end of the chapter is most inaccurate (Pflüg. Arch., vol. iv, p. 131). Traube's name does not appear as one of the contributors of the first 124 volumes (1908) of Pflüger's Archiv. We have not, after days of search assisted by a most able librarian, been able to find any article by Traube on the subject of vomiting, whether in Pflüger's Archiv or in any other scientific journal.

[4] O. Hesse: Zur Kenntnis des Brechaktes. Arch. f. gesam. Physiol., vol. 152, June, 1913, pp. 1–22. Hesse reprints one of Guinard's tracings.

[5] Louis Guinard: Étude Experimentale de Pharmacodynamie comparée sur la Morphine et l'Apomorphine. (These) Faculté de Médecin et de Pharmacie de Lyon, Tome 10, 1897–1898.

The tracings in this article are as nearly identical with some of our own as blood pressure tracings could well be. To Guinard, therefore, belongs the credit for priority in this field. We are sorry that we failed to discover his work sooner; but since his research was a thesis not published in any accessible journal it escaped notice. In this paper we wish to call attention to this work confirmed by us, and to extend the work by adding experiments which throw some light on the mechanism which is responsible for the results obtained by him and ourselves.

Though E. Harnack[6] many years before Guinard purported to make a study of blood pressure and pulse rate during vomiting induced by apomorphine an examination of his protocol-tables shows that at the exact moment of emesis and for some minutes afterward (7–20 min.) he took no record of the blood pressure or the pulse rate. As a result he failed to discover the remarkable phenomenon first described by Guinard and confirmed by our work. His experiments deal with blood pressure phenomena preceding and following the injection of apomorphine but he has no data on the blood pressure and pulse rate at the moment of expulsion of the vomitus.

EXPERIMENTAL METHODS

The methods employed in these experiments are those previously devised for study of blood pressure on the quiescent unanesthetized animal.

The T-cannula was inserted into the carotid under ether anaesthesia and several hours later or the following day, when the animal had recovered from the effects of the anaesthetic, the studies on the blood pressure during vomiting were made. Several additions have been made in the technique used lately. These briefly described are as follows:

First is the tambour piston separation cannula. This instrument is a modification of the old T-glass cannula. It consists of a metal cannula whose side arm is relatively of large calibre

[6] E. Harnack: Arch. f. exp. Path. und Pharm., vol. 12, 1874, p. 254. See tables on pp. 261, 262, and 263.

and contains a piston consisting of a hollow hard rubber cylinder with a thin rubber diaphram over one end. This rubber diaphram can give way and thus permit the column of liquid to transmit the impulses from the heart to the mercury manometer with slight damping of the curve. For the larger changes in the blood pressure the piston itself can slide up or down following the pull of the rubber diaphram. The advantage of the diaphram separation cannula is, that it separates practically completely the blood from the fluid in the manometer tube and therefore prevents the entrance of toxic substances into the circulation which constitutes a difficulty in work done upon the quiescent animal. The second is an oil separation cannula. The object of the instrument is the same as that of the rubber diaphram separation cannula; but here the separation is accomplished by means of an inverted U-tube filled with oil which floats upon the blood column in one arm, and the fluid which is used to connect with the mercury manometer of the other arm. This accomplishes the separation without the interposition of any diaphram and is therefore without the slight damping effect observed in the diaphram cannula.

A third addition to the technique is the injection of a sufficient amount of leech extract into the cannula at intervals to prevent clotting. The leech extract is prepared by the method of Abel, Rowntree and Turner,[7] or by grinding up the heads of leeches in a mortar, extracting for 24 hours in water, precipitating the proteins by heat and dilute acetic acid and filtering and neutralizing with sodium bicarbonate.

The experiments were performed upon dogs. Vomiting was induced by digitalis, apomorphine subcutaneously, and by zinc sulphate, magnesium sulphate by the mouth. Different types of animals were employed for different experiments; for example, small wiry muscular fox terriers were employed for studying the effects of the muscular contractions of the abdomen and other skeletal muscles concerned in vomiting, and on the other hand

[7] Abel, Rowntree and Turner: On the Removal of Diffusible Substances from the Circulating Blood of Living Animals by Dialysis. J. Pharm. and Exp. Therap., vol. v., no. 3, Jan., 1914, pp. 275-316.

young adults or half-grown pups with large abdomens were used to study those phenomena of vomiting that are brought about by the gastric contraction. Observations on the respiratory movements were made by inspection and in some instances by tracing the movements by means of a tambour.

EXPERIMENTAL RESULTS

Our results indicate that there are different types of vomiting, the variations depend principally upon the character of the drug employed, or upon the consistency of the gastric contents or the degree of participation of the skeletal musculature or of the diaphragm or the gastrointestinal musculature respectively.

In general there are two types of vomiting.

First there is the rapid projectile type, characterized by suddenness of onset, with slight premonitory symptoms, and with apparently a preponderance of the gastro-intestinal tract activity over the activity of the skeletal muscular system.

Second there is the slow labored type of vomiting characterized by rather marked prolonged premonitory symptoms and with apparently more of the skeletal muscles taking part in the process which consists of prolonged violent retching movements, meanwhile there are great oscillations of the blood pressure, and which ends with vomiting followed by exspiration and a noise just after the vomitus is expelled. Or to summarize:

First type. Rapid projectile. Preliminary nausea with slight rise in blood pressure and with a decrease in amplitude of pulse wave, and with an increase in pulse rate. Vomiting is accompanied by a sharp fall in the blood pressure, during which there is slowing or almost cessation of the heart beat, or arrhythmia. Respiration is almost completely suspended so far as ventilation of the lungs is concerned for the epiglottis is closed The vomitus is expelled near the low point of the blood pressure curve (fig. 1) and immediately after there is a return of respiration and a recovery of the blood pressure which rapidly mounts to the normal or even above the normal.[8]

[8] Dr. A. J. Carlson observed a drop in blood pressure and slowing of heart in an unanesthetized dog when vomiting was induced by the inhalation of ozone.

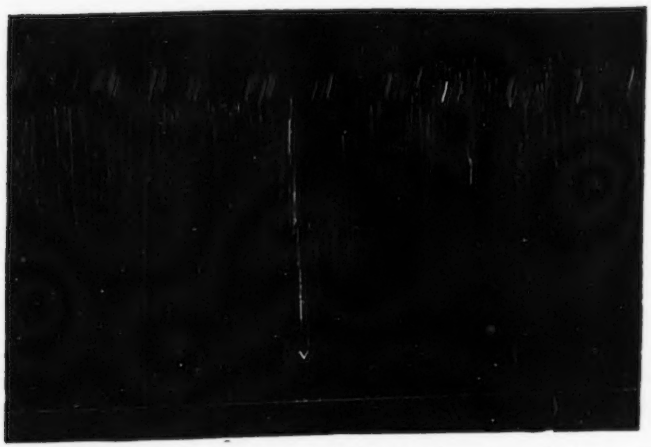


Fig. 1. Blood-pressure tracing showing fall in blood-pressure during vomiting. The vomiting is of the quick projectile type. "V" shows the point where the vomitus was thrown out. Time is recorded in seconds.

Fig. 2. Type of tracing obtained during quick projectile vomiting where atropine had been previously administered. Vomitus was expelled at 9, 10, and 11.

Second type. Slow labored type. Preliminary nausea, deep breathing, salivation. Then comes on violent retching with great oscillations of blood pressure. At this time the pulse beats are irregular and slow. Respiration is suspended so far as the ventilation of the lungs is concerned but there are violent changes in the intrathoracic pressure which correspond with the retching movements. After a long series of retching movements, the vomitus comes up into the pharynx at the instant the top of one of the violent blood pressure oscillations is reached (fig. 3). The animal then takes breath and the blood pressure after suddenly falling again during the inspiration mounts to the normal or above the normal.

Vomiting after the administration of atropine

It was thought that since the vagus center and the central nervous areas that are concerned in vomiting, are adjacently located in the central nervous system, that it was very probable that the fall in blood pressure and the slowing and the irregularities in the pulse rate during vomiting are brought about through the vagus mechanism. Evidence in favor of this view was obtained by first making the observation of vomiting on the normal dog, and then later on the same dog, making a second observation of vomiting after the administration of atropine. It was found that after the administration of sufficient atropine to paralyze the vagus endings of the heart, that the vomiting failed to produce the marked fall in blood pressure, the amount of slowing of the heart, nor the degree of arrhythmia, as had been observed in the same animal previous to the administration of the atropine (fig. 2).

Vomiting after the administration of curare

It was thought that the great oscillations of blood pressure noticed during the violent retching movements seen in the second type of vomiting were due chiefly to the action of the skeletal muscles in the violent contractions, as well as their effects by pressure on the abdomen and vacuum formation in the thorax.

Fig. 3. Blood-pressure tracing during vomiting of the slow labored type. Here violent retchings continue for a long time. The tracing begins several seconds after the onset of retching. At the points marked "V" vomitus was expelled and some breath taken. At the left end of the chart the second round of the blood-pressure tracing is lapped for a few inches in order to show the height of the pressure as compared with that of the first round which was taken during the retching period.

Fig. 4. Blood-pressure tracing showing a reduction of retching movements in the slow labored type of vomiting. This reduction was caused by a small dose of curare. On the left end of the chart the second round of the tracing continued for a short distance lapped over the first round in order to show the comparative height of pressure during the retching period and shortly after vomiting. 1, 2, retching. V, vomiting. 3, recovery.

Therefore it was further thought that if this be true, then after more or less complete paralysis of these skeletal muscles by means of curare, vomiting should show a corresponding decrease in these osci lations of the blood pressure.

It was found that after the subcutaneous administration of curare in sufficient quantities to have a marked depressing effect upon the animal, but not sufficient to cause complete immobility then vomiting failed to produce the violent retching, and likewise failed to produce the great oscillations of the blood pressure (fig. 4). The conception of the mechanism involved is as follows:

1. Vagus inhibition of the heart is an important factor in the production of the lowering of the blood pressure, inhibition of the hea t, and arrhythmia noticed during vomiting. In an animal in which the cardio-inhibitory nerve endings have been paralysed with atropine these blood pressure changes just mentioned are reduced.

2. Muscular contraction *per se* causes an increase in the blood pressure. The violent contractions of the skeletal muscles seen during retching always cause an increase in the blood pressure, and there is a fall in the blood pressure during the periods of relaxation which intervene. This rise in the general blood pressure observed during retching can be diminished by partly curarizing the animal.

SUMMARY

Vomiting is accompanied by marked changes in the circulatory system and interference with respiration. There is seen sometimes a period of elevated pressure, more frequently a sudden and enormous drop in blood pressure with cardiac inhibition at the moment of emesis, always periods of great oscillations in the blood pressure. These great and sudden oscillations of the blood pressure may cause a rupture of the blood vessels which would not occur with the same pressure but with slower changes. The faint feeling accompanying emesis may be the result of the cerebral anemia occasioned by the cardio-inhibition and resulting drop in blood pressure.

THE EFFECT OF THYROID EXTRACTS UPON BLOOD PRESSURE[1]

G. G. FAWCETT, JOHN ROGERS, JESSIE M. RAHE, S. P. BEEBE

From the Department of Experimental Therapeutics, Cornell University Medical College, New York

Received for publication October 24, 1914

The function of the thyroid gland is probably dependent upon the production of certain substances constituting an internal secretion which reaches the tissues through the medium of the blood and lymph. It is reasonably certain that the active portions of this secretion can be obtained from the gland by various methods of extraction. The effects upon the blood pressure of extracts from the various glands of internal secretion have been tested in numerous laboratories during the last twenty years.

The first kymograph experiments to determine the effect of intravenous injections of aqueous extracts of the thyroid were made by Oliver and Schafer (1) who obtained a marked fall in blood pressure. They found that extracts of the fresh glands were somewhat more active than those made from old or dried material. A glycerin extract was no more effective than the aqueous preparation. Boiling the extract did not alter its depressant effect which apparently resulted from its action upon the heart. Oliver and Schafer found also a similar blood pressure depressing effect to follow the intravenous injection of extracts made from many other organs.

Haskovec (2) experimented with extracts of the thyroid similarly prepared and in the main confirmed the previous findings. He, however, obtained a slightly increased frequency of the pulse rate which seemed to be due to central stimulation of the cardio accelerator nerve. He attributed the fall in blood pressure to a

[1] The expenses of this investigation were defrayed by the Johnston Livingston fund for Experimental Therapeutics.

direct action upon the heart muscle. He experimented also with alcoholic extracts of the thyroid gland and found that intravenous injection of these extracts produced no change of blood pressure.

Georgiewsky (3) experimented with thyroid extracts prepared in a similar manner and found essentially the same results but Guinard and Martin obtained a retardation instead of acceleration of the pulse rate.

Fennyvessy (4) found the fall in pressure to occur after section of the vagus and after the administration of atropin and considered it due to a peripheral effect of the extract.

Svehla (5) could obtain no effect from the injection of extracts of embryonic thyroids and only slight effect from those derived from the glands of new born animals.

Several other investigators contributed confirmations of the results of these last two investigators.

Heirratz (6) and Livon (7) claimed from their experiments with aqueous extracts of the thyroid an elevation of blood pressure.

More recently Asher and Flack (8) injected intravenously measured quantities of alkaline aqueous extracts of fresh and dried thyroids and could detect little or no effect upon the blood pressure.

In contradiction to these results Popielski in 1911 (9) isolated from various organs, including the thyroid, a substance which he called vasodilatin which substance he claims is in no way identical with choline or β-imidazolylethylamine and is the active principle of organ extracts. This substance was obtained by digesting the proteins of the glands with pepsin and trypsin. Details of the method are not given. Popielski claims that his vasodilatin diminishes blood pressure, produces incoagulability of the blood and favors the secretory activity of the glands. This diminished blood pressure is not brought about by change in heart action but by direct dilatation of the blood vessels.

Lampe, Liesegang and Klose (10) experimented with alkaline extracts of human thyroid, both normal and goitrous, and obtained only a slight fall in blood pressure without acceleration

of the pulse. But extracts of the fresh thyroids obtained from operated cases of Graves disease injected into nervous fox terriers produced a fall in blood pressure of 20 to 40 mm. of mercury, accelerated the heart action and raised the temperature. A repetition of their experiments by O. Bardenhewer (11), however, failed to confirm these findings.

It is interesting to note, however, in conjunction with the seemingly constant presence of a vasodilator substance in normal thyroids and the probable excess of thyroid secretion in conditions of hyperthyroidism, some recent experiments of Black and Sanford (12). They obtained serum from patients suffering from more or less pronounced Graves disease, and found after its intravenous injection into dogs a drop in blood pressure which corresponded in some degree to the severity of the process in the patient from whom the serum was obtained. Control experiments with serum from nervous subjects without evidence of hyperthyroidism were negative. Extracts made from diseased thyroids not of the exophthalmic goitre type removed by operation produced some fall in blood pressure but it was not as marked as the fall obtained from extracts of the glands of pronounced Graves disease. They state that a repetition of the injections in the same animal seemed to establish a tolerance. The second and third injections during the usual experimental period produced less effect than the first.

V. Fürth and Schwartz (13) have attempted to prove that the vasodilator effects which have been noted after the injection of extracts of practically all organs and tissues are due to the presence in them of the same substance; namely, cholin. This conclusion is very probably erroneous, as shown by Vincent (14).

It is evident that aqueous extracts of the thyroid may contain more than one active substance. Frankel (15) extracted the gland in boiling water and after removing the albumen obtained a crystalline body, having the formula $C_7H_{11}N_3O_3$. But this contains no iodin, and though it was said to have produced hyperthyroid symptoms after subcutaneous injection, no kymograph tracings were made.

Baumann (16) produced so called iodothyrin by boiling the

thyroid in 10 per cent sulphuric acid. This substance contains a high percentage of iodin and is used therapeutically but numberless experiments have proved that at least it is not the normal active ingredient of the organ.

Oswald (17) has attempted to show that iodothyrin is derived from a preceding thyroglobulin in the colloid. All investigators are practically agreed that whatever the active ingredient of the thyroid may be, it has a definite relation to the content of iodin.

A comparison of the results of injections of thyroid extracts and of iodothyrin into the circulation shows quite marked differences with different observers but the weight of evidence is against any noticeable fall of blood pressure after intravenous injections of iodothyrin. It, nevertheless, seems to show rather constant effects upon the nerve supply of the heart, the details of which appear to vary in different animals. von Cyon and Oswald (18) state that it stimulates the cardio-inhibitory center and increases the electric excitability of the vagus and affects the terminal filaments in the heart muscle. von Fürth and Schwartz (13) claim that it has a direct effect upon the heart muscle. Barbera (19) compares these results with injections of iodin and its salts, and seems to show that the latter have an antagonistic action to iodothyrin. Iodin and its salts apparently paralyzed the vagus and its nervus depressor, and excited the cardiac and vascular sympathetic nervous system. After injections into the circulation of iodothyrin the electric irritability of the vagus and sympathetic nerves is increased. The salts of iodin have an opposite effect, but seem to directly stimulate the sympathetic and produce in dogs and rabbits an increase in temperature, blood pressure and pulse rate. We have not been able to confirm these findings. von Cyon (20) has attempted to prove that the electric irritability of the nervus depressor after the injection of iodothyrin is greatly increased. Asher and Frank (21) have assumed that von Cyon's statements are correct and that the secretory nerve fibres for the thyroid are derived from the superior and recurrent laryngeals, and claim that electric stimulation of the nervus depressor shows much more pronounced effects after than before a preceding stimulation of the laryngeals. In other words, that an increased output of thyroid

secretion produced by stimulation of the secretory nerve supply of the gland, sensitizes the nervus depressor. If the thyroid is removed and the laryngeals then stimulated, no increased excitability in the nervus depressor is demonstrable. They also state that after stimulation of the laryngeals, which is supposed to produce an increase in the thyroid output, an injection of adrenalin produces a higher rise in glood pressure than a similar injection made when the laryngeals have not been stimulated.

Kraus and Friedenthal (22) state that mixing adrenalin with extracts of thyroid prolongs and intensifies the action of adrenalin.

From the experiments quoted it is quite definite'y established that extracts of the entire thyroid gland contain a substance or substances which have a pronounced effect on lowering blood pressure. These effects are more pronounced when obtained from fresh rather than dried glands and from those from mature rather than young animals. It is also clear that boiling does not destroy the vasodilator property. The effects upon pulse rate and respiration are at least insignificant.

Our experiments have been begun with an attempt to isolate from a watery extract of the fresh gland all of the substances which can be separated with as little alteration as possible of their chemical structure. These include the socalled nucleoproteins, globulins, coagulable and non-coagulable albumens, and the alcohol soluble and alcohol insoluble residue. Each of these bodies has been dissolved and injected intravenously in dogs, and the results upon the blood pressure, respiration and heart action noted in the usual kymograph tracings. The solution of the alcohol soluble residue left after removal of all other substances has been found to be the only substance which produces any appreciable effect upon the blood pressure.

The glands used throughout these experiments have been pig thyroids. These were chosen because of their relatively high iodine content over those of the beef or sheep. It has also been found that the separation of the nucleoproteins from the extract of the pig glands was readily brought by precipitation with acetic acid without warming to 44°C. as required by the extracts from sheep and beef glands.

PREPARATION OF THE VARIOUS THYROID EXTRACTS USED IN THE EXPERIMENTS

The thyroid glands were obtained from a slaughter house in as fresh a condition as possible. They were trimmed free from any adhering fat or connective tissue and immediately finely ground up in an ordinary meat grinder. This hashed up material was then placed in large, wide-mouthed bottles with twice its volume of physiological salt solution made faintly alkaline with sodium hydroxide. It was shaken for two hours and then placed in a refrigerator over night.

After this extraction the liquid was filtered off, first through a double thickness of cheesecloth and then through paper pulp in a Büchner funnel. The extract was now ready for separation.

I. The nucleoproteins were precipitated by acidifying with 10 per cent acetic acid. After standing for half an hour or so they settled out and were filtered off. The proteid precipitate was ground up, suspended in distilled water, dissolved with 10 per cent sodium hydroxide, filtered, and reprecipitated with 10 per cent acetic acid. After settling out the nucleoproteins were washed by decantation five or six times with distilled water. After being again suspended in water and dissolved with as little 10 per cent sodium hydroxide as possible, they were ready for use.

The acid filtrate from the first precipitation was the basis of all the remaining proteins used.

II. The globulins were obtained by half saturation of this acid filtrate with ammonium sulphate. These were filtered off, dissolved in distilled water, placed in parchment paper dialysing bags and dialysed for ten days or until there was no sulphate shown to be present.

III. The filtrate from the globulins was saturated with ammonium sulphate and the albumins so obtained were filtered off and dialysed exactly as the globulins.

IV. The coagulable proteins were obtained by boiling the acid filtrate, filtering off the coagulated portion, making faintly alkaline with sodium hydroxide, boiling again and again filtering.

The combined precipitates so obtained were washed several times with distilled water and then dissolved with as little sodium hydroxide as possible.

V. The protein substance that is here called the "residue" was the filtrate remaining after all the coagulable proteins had been removed. This was always used in a slightly alkaline condition.

VI. The residue was evaporated to as near dryness as possible and found to be partly soluble in 95 per cent alcoho'. This gave the two preparations called "alcohol soluble" and "alcohol insoluble." The alcoholic solution was evaporated to drive off the alcohol and the residue remaining was taken up with distilled water in which it was readily soluble, and made just alkaline with sodium hydroxide. The alcohol insoluble portion was dissolved in water and made faintly alkaline in the same manner.

VII. The portion "alcohol soluble" was further fractionated by completely precipitating the peptones with basic lead acetate. This gave the two portions called "lead precipitate" and "lead filtrate."

The lead precipitate was washed several times with distilled water to free it of the filtrate, made slightly acid with acetic acid, heated and decomposed by passing sulphuretted hydrogen gas through it. After complete precipitation of the resulting lead sulphide, it was filtered and the filtrate evaporated almost to dryness, taken up again with distilled water, filtered and made faintly alkaline before using.

The lead filtrate was treated similarly with sulphuretted hydrogen gas to precipitate any excess of the basic lead acetate, filtered, evaporated to approximate dryness, taken up with distilled water, filtered again and made faintly alkaline for use.

VIII. Some iodothyrin according to Baumann's process was obtained and a saline extract of this prepared for comparative work as was also physiological salt solution extract of some "Desiccated Thyroid Glands" prepared by Parke, Davis & Co.

The protein content of each of these preparations was obtained by finding the per cent of nitrogen by the Kjeldahl method and multiplying this by the factor 6.25. The iodine content was

obtained by Rigg's modification of the Baumann process with the further slight modifications given in a previous paper. Whenever two or more of the various preparations were made from different sets of glands, the ratio of the nitrogen and iodine in each was found to be fairly constant and any variation can be accounted for under seasonal variation if not merely within the limits of unavoidable technical errors.

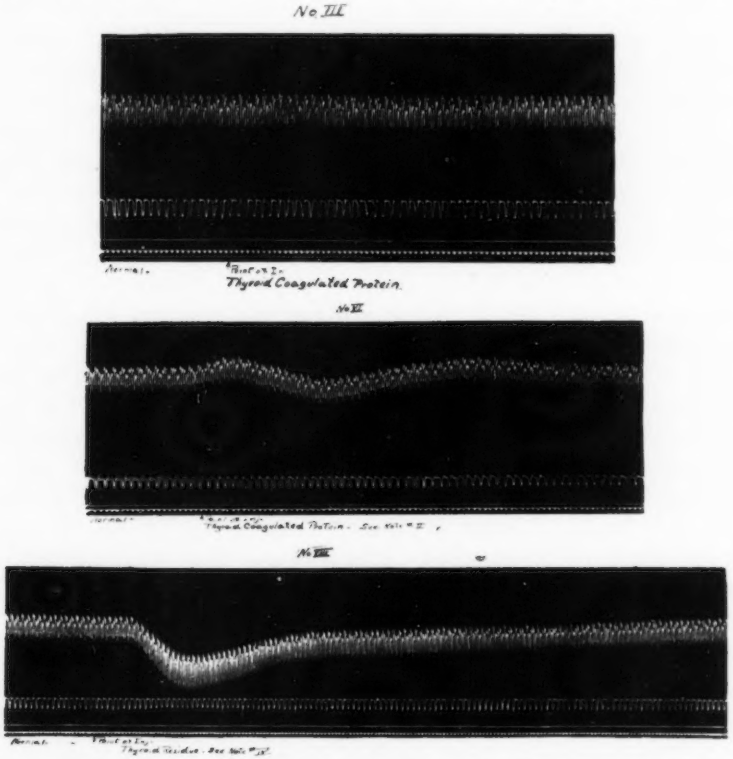
Further work in regard to the chemical nature and properties of this "residue" is being carried on and will be published in subsequent papers.

The following table shows the nitrogen and iodine content of the extracts used:

	PER CENT OF PROTEIN	MGS. OF IODINE PER CC.
Nucleoproteins	1.860	0.12000
Globulins	0.700	0.01360
Albumins	0.175	Trace
Coagulable Proteins	0.800	0.01140
"Residue"	4.025	0.02785
Alcohol Soluble	5.820	0.00880
Alcohol Insoluble	2.700	0.01040
Lead Precipitate	0.660	Trace
Lead Filtrate	2.660	0.00050
Iodothyrin	0.407	0.00015
Parke, Davis & Co., desiccated thyroid	0.500	0.02400

The proteins described in the previous pages were used in an extensive series of experiments to determine their effect upon blood pressure, pulse rate and respiration. In the preliminary experiments we were struck with the marked fall in blood pressure produced by the non-coagulable portion of the extract called "Thyroid Residue." This was so marked in its effect that we were led to compare all the other fractions to it giving comparable doses in each instance. It was further found that the depressant substance was located in the alcohol soluble portion of the residue; also in the filtrate from the lead precipitate. It was further demonstrated that the depressant effect of the various proteins bore no relation to the content of nitrogen and iodin.

Dogs were the experimental animals used throughout the experiments. The injections were all made in the femoral vein, blood pressure taken from the carotid artery. Before the injections were begun numerous experiments were made to determine the effect of anesthesia on the blood pressure and it



was found possible to keep the dogs under ether for periods of three or four hours without changes in the blood pressure.

In striking contrast to the effect obtained by intravenous injection are the results showing that the subcutaneous injection of enormous doses of the most active preparation obtained produced no effect upon the blood pressure. The experiments were varied in some instances, giving comparable doses of nitrogen and in other instances comparable doses of iodin but the results

were absolutely uniform in showing that the complete proteins did not produce depressing effects in any way comparable to the effects produced by the alcohol soluble and lead filtrate portions of the residue. Enormous doses of the protein produced only relatively slight reactions. The intravenous injection of the active portions of the residue never failed to produce a reaction when the blood pressure in any degree approached normal. After the injection of large doses of the proteins without effect, a small dose of the residue produced a prompt response.

In all experiments were made upon seventy-five dogs. The tracings which are published were taken from three animals but they are identical with those obtained in a large series. The recovery of the animal following a small injection of the active portion of the residue is prompt as will be seen from the tracings. The injection of large doses of residue caused a depression in blood pressure nearly to the zero line; the recovery to normal in such cases was very gradual but finally complete.

On account of the limited space, only three of the typical tracings will be produced. Tracing No. III, showing the effects of the Thyroid Coagulated protein, with its protein dosage equivalent to that of the Residue, in tracing No. VIII, and tracing No. VI with its protein and iodine dose many times stronger than that of the Residue. Tracing No. VIII is typical of the effects of the Residue. The effects of the remaining preparations from the Thyroid are given in Plates I and II in tabulated form. The remaining tracings are filed with this JOURNAL.

SUMMARY

1. Iodine is present in all the fractions of the extract.

2. The coagulable fractions, which are the richest of all the fractions in iodin, produce little or no depressor effect even in relatively enormous dosage.

3. The "Residue," or fraction of the extract which remains after the removal of the coagulable fractions, contains iodin but in smaller quantity than the other fractions.

4. The depressor effect of the "Residue" bears no relation to the total iodin content of the gland from which the residue is derived.

	Tracing Number	I	II	III	IV	V Note #I	VI Note #II	VII Note #III	VIII Note #IV	IX Note #V	X	XI Note #VI	XII Note #VII
	Thyroid Solution	Residue	Globulin	Coagulated Protein	Albumin	Globulin	Coagulated Protein	Albumin	Residue	Residue	Nucleo-protein	Thyreo-iodin	Residue
Protein	Mgms. per c.c. Solution	40.25	7.	8.05	1.75	7.	8.05	1.75	40.25	40.25	18.6	4.07	20.
Iodine	Mgms. per c.c. Solution	.02785	.0136	.0114	Trace	.0136	.0114	Trace	.02785	.02785	.12	.00015	.0185
Dosage Intra-venous.	Minims	5.	26.3	22.9	105.6	263.	320.	528.	5.	5.	10.	240.	8.6
	Mgms. Protein	11.55	11.55	11.55	11.55	115.5	167.	57.75	11.55	11.55	11.55	183.	11.55
	Mgms. Iodine	.0087	.02235	.016316	Trace	.2235	.2274	Trace	.0087	.0087	.075	.00675	.0106
Effects on Blood Pressure	Fall in Mms. Mercury	32.	0.	0	0	Secondary Fall 8 mms.	Second-ary Fall 10 mms.	Second-ary Fall 12 mms	38.	49.	0	0	22.
	Rise in Mms. Mercury	0.	0.	0	6.	Primary Rise 4 mms	Primary Rise 5. mms	Primary Rise 6 mms	0	0	0	0	0
	Latent Period before Effect.	4. sec.	0.	0	13. sec.	2. sec.	2. Sec.	27. sec	9. sec.	4. sec.	0	0	4. sec.
Effects on Heart Rate	Rate per min. before injection	144	150	138	138.	141	144	144	138.	205	198	198	216
	Rate during time of Action	144	—	—	138.	141	144	144	See Note #VIII	204	—	—	210
	Rate after Bld. P returned to Normal	144	150	138	138	141	144	144	138	204	198	198	210
Time of Action		2 min.	0	0	2 min. 49 sec.	40 sec.	1 min. 20 sec	2 min.	2 min. 49 sec.	1 min 44 sec	0	0	49 Sec
Respiratory Effects		0	0	0	0	0	0	0	0	0	0	0	0

Notes and Remarks.

#I Ten times the protein dose and 25.5 times the Iodine dose of Residue.
#II 14.5 " " " " " 26.1 " " Iodine was present only in slight trace.
#III 5.
#IV Residue inj. to check Bld P. condition on dog #1.
#V New dog. #II.
#VI This is the Thyreoiodin prepared according to "Baumann".
#VII Third dog and new solution of Residue. Protein dose same as in Tracing #I Iodine .0019 mgms greater.
#VIII At lowest point of curve of Bld. P. Pulse rate 126 per min. at middle of rising Bld. P Pulse rate 132 per min.

C.E. Fawcett

	Tracing Number	XIII Note #VIII	XIV Note #IX	XV Note #X	XVI Note #XI	XVII Note #XII	XVIII	XIX[1]	XIX[2]	XIX[3]	XIX[4]	XX
	Thyroid Solution	Residue Alcohol Soluble	Residue Alcohol Insoluble	Residue Pb Filtrate	Residue Pb Ppte.	Merke Dan's Dessicated Thyroid	Same as XVII	Residue Note #VIII				Residue Note #XIV
Protein	Mgms. per c.c. Solution	58.2	27.	26.6	5.	5.	5.	20.				20.
Iodine	Mgms. Per c.c. Solution	.0088	.0104	.0005	Trace	.024	.024	.0185				.0185
Dosage Intra-Venous	Minims	18.	64.	6.3	160.	34.65	160.	160.				8.6
	Mgms. Protein	69.8	115.5	11.55	53.3	11.55	53.3	213.				11.55
	Mgms. Iodine	.0106	.0443	.00021	Trace	.0561	.256	.1973				.00106
Effects on Blood Pressure	Fall in Mms. Mercury	40.	12.	19.	18.	0.	12.	83.				40.
	Rise in Mms. Mercury	Secondary 12.	0	0	0	0	0	0				0
	Latent period before effect	6.5 sec	6 Sec.	5 Sec.	5 Sec	0	4.5 sec.	4 sec.				6.5 sec
Effects on Heart Rate.	Rate per Min. before Injection	204.	186.	186.	180.	180.	180.	186.				216.
	Rate during time of action	235.	174.	186.	180.	—	178.	Lowest Point 128	234.	216.	198.	216.
	Rate after Bld. P. Returned to Normal	240.	186.	192.	174.	180.	180	190			190.	216.
Time of Action		2 Min.	62 Sec.	49 sec	40 sec.	0.	50 sec.	32 min.				76 sec.
Respiratory Effects		0	0	0	0	0	0	Resp. Shallow	Slightly Slowed	Slightly Slowed	Normal	0.

Notes and Remarks.

#VIII A dose with same Protein content as the Residue, gave no effect, Similarly with Iodine.
#IX Inactive in doses corresponding to Prot. and I. content of Thyroid Res. Dose inc. Ten times.
#X Iodine content of dose much smaller than that of Res.
#XI Only a Trace of Iodine could be detected. Enormous dosage was required to give any effect on Bld. Pressure.
#XII The Protein and Iodine content of the solution, prepared from the commercial preparation was proportionately the same as the Prot. and I. content of the Dessicated Thyroid.
#XIII Tracings #1-2-3- of XIX are consecutive #4 is taken when Bld. P. is about normal. The large dose was given to test the toxic effect of the Residue. Time between 3 and 4 = 22 min. 31 sec, in which the rise of Bld. P. was gradual and heart action regular.
#XIV The basic dose of Residue, was given directly after the large dose #XIX to check the condition of Bld. P. and Heart action.

G.G. Fawcett

5. The depressor effect of the "Residue" follows only after its intravenous injection. It is not manifested after subcutaneous injection.

6. Tachycardia is not regularly produced by this depressor substance although a very large dose (10 cc.) does accelerate the pulse rate at the lowest points of blood pressure. Later the force and rate of the heart beat return to normal with the return to normal of the blood pressure. (See tracings XIX[1], XIX[2], XIX[3], XIX[4].

7. The active portion of the "Residue" is not precipitated by basic lead acetate; is not altered by boiling nor by passage through a Berkefeld filter; and is soluble in alcohol.

8. No immunity to the depressor effect of the "Residue" or of its active portion was noted during the limit of the experiments (4–6 hours).

REFERENCES

(1) Oliver and Schafer: Journal of Physiol., 1895–1896, xviii, 276.
(2) Haskovec: Wien, Med. Blät, 1896, xix, 115, 166.
(3) Georgiewsky: Zentral f. d. Med. Wissensch., 1895, xxxiii, 456.
(4) Fennyvessy: Wien. klin. Wochs., 1900, xiii, 125.
(5) Svehla: Archiv f. Exp. Path, und Pharmacal., 1900, xliii, 321.
(6) Heirratz: "Das alte und das neue über die Schildrüse." Dissertation 1894.
(7) Livon: Compt. Rend. Soc. di Biol., 1898, v, 98.
(8) Asher n. Flack: Zeits. f. Biol., 1910–1911, lv, 83.
(9) Popielski: Arch. f. die Ges. Physiol., 1911, cxxviii, 222.
(10) Lampe, Klose und Liesegang: Beit. z. Klin. Chir., 1912, lxxvii, 601.
(11) Bardenhewer: Arch. f. Klin. Chir., 1912, xcvii, 729.
(12) Black and Sanford: Journal American Medical Assoc., 1914, lxii, 117.
(13) Von Furth and Schwartz: Archiv f. d. Ges. Physiol., cxxiv, 361.
(14) S. Vincent: Internal Secretions and the Ductless Glands. E. Arnold, London, 1912.
(15) Frankel: La Semaine Medical, 1895, p. 511.
(16) Baumann: Zeits. f. Physiol. Chem., 1896, xxii, 1.
(17) Oswald: Zeits. f. Physiol. Chem., 1910, lxviii, 173.
(18) V. Cyon and Oswald: Arch. f. d. Ges. Physiol., lxxxiii, 199.
(19) Barbera: Archiv f. d. Ges. Physiol., 1900, lxix, 312.
(20) Von Cyon: Die Gefassdrusen als Regulatorische Schutzorgone des Zentralnervensystem, Berlin, Springer, 1910.
(21) Asher and Frank: Zeits. f. Biol., 1910–1911, iv, p. 83.
(22) Kraus und Friedenthal: Berl. Klin. Wochenschrift., 1908, xxxviii, 604.
(23) Riggs: Amer. Journ. Physiol., xxxiv, No. 1.

THE INFLUENCE OF OXALATES, CITRATES AND TARTRATES ON THE ISOLATED HEART

WILLIAM SALANT AND SELIG HECHT

From the Pharmacological Laboratory, Bureau of Chemistry

Received for publication November 4, 1914

The action of salts forming insoluble calcium compounds has frequently been explained by their physico-chemical properties. Disturbance of the calcium mechanism was, with very few exceptions, urged as the only cause underlying the changes produced by the soluble salts of oxalate, citrate and tartrate when brought in contact with the living cell. According to one view, which may be considered the dominant theory, this is accomplished by the precipitation of calcium, while another explanation assumes that the action may be due to the transformation of calcium into a non-ionized condition. The evidence on which these theories were based was derived by various methods of inquiry. Marked disturbance of calcium metabolism following the administration of soluble oxalate to animals is one of the arguments employed in favor of the contention that the mode of action of this salt consists in depriving the body of this important metal. According to Caspari,[1] who experimented on dogs, and Luitblein,[2] who employed rabbits, the elimination of calcium is greatly increased after the administration of sodium oxalate. Corroborative evidence of decalcification was furnished also by the chemical studies of Sarvonat and Roubier,[3] who analyzed the skeleton and soft parts of three guinea pigs which had been receiving sodium oxalate for 6 to 7 days. The amounts of calcium found, according to their observations, were much reduced as a result of the treatment, the soft parts being affected before the

[1] Caspari: Centralblatt f. Agricultur Chemie, 1897, **26**, p. 529.

[2] Luithlein: Archiv für experimentelle Pathologie u. Pharmakologie, 1912, **69**, p. 375.

[3] Sarvonat and Roubier: Comptes Rendus Societe de Biologie, 1911, **71**, p. 104.

bony tissues. It is interesting to note in this connection that according to Dixon's[4] observations the prolonged administration of oxalates to rabbits resulted in changes in the bones resembling rickets. The observations of Loew,[5] who reported that oxalic acid is not toxic to molds which grow without calcium, have been looked upon as especially favorable to the calcium theory.

But the main support for the contention that the calcium of the tissues is in some way concerned in the changes produced when they are acted upon by the salts under consideration was furnished by the results of the experiments indicating antagonistic action when the tissues are exposed to the combined influence of any of these compounds, and of soluble lime salts. The data which form the basis for this theory were obtained by observations under a variety of conditions and include experiments on isolated organs, as well as on the intact animal. According to the observations of Howell,[6] which were corroborated later by Locke,[7] the irritability of the gastrocnemius of the frog which is immersed in sodium oxalate may be restored by means of calcium. Januschke[8] reached the same conclusion in experiments with sodium oxalate on the isolated heart of the frog.

Recently Gates and Meltzer[9] reported experiments with sodium oxalate and magnesium sulphate in which it was shown that the action of magnesium sulphate is much more toxic when oxalate is administered at the same time. This was assumed to be due to the precipitation of calcium which, as previously shown by Meltzer and Auer,[10] antagonizes the action of magnesium. In a later communication Gates and Meltzer[11] demonstrated that the

[4] Dixon, W. E.: Manual of Pharmacology, p. 344, London, 1906.

[5] Loew: Biochemische Zeitschrift, 1912, **38**, p. 226.

[6] Howell: Journal of Physiology, 1894, **16**, p. 476.

[7] Locke: Journal of Physiology, 1894, **15**, 118.

[8] Januschke: Archiv für experimentelle Pathologie u. Pharmakologie, 1909, **61**, p. 363.

[9] Gates and Meltzer: Proceedings of the Society for Experimental Biology and Medicine, 1913, **11**, p. 23.

[10] Meltzer and Auer: American Journal of Physiology, 1908, **21**, p. 400.

[11] Gates and Meltzer: Proceedings of the Society for Experimental Biology and Medicine, 1914, **11**, p. 97.

anesthesia and paralysis produced by subminimum doses of sodium oxalate and of magnesium sulphate may be promptly abolished by the administration of calcium chloride. The same conclusions were reached by other observers who studied the action of these salts on the intact animal as well as on isolated organs. Froelich and Chiari[12] observed increased irritability of the sympathetic system by the injection of sodium oxalate. This was shown by the greater mydriatic effect of adrenalin and by a rise of blood pressure which were readily counteracted by means of calcium chloride. According to Decottignie[13] the toxicity of sodium oxalate may be decreased by the previous administration of calcium chloride or glycero-phosphate of calcium, so that even a surely fatal dose of the oxalate may be thus survived. Sugimoto's[14] experiments on the isolated uterus of the guinea pig have likewise furnished evidence of this antagonistic action. When 5 mgm. of sodium oxalate were added to 10 cc. Ringer's Solution containing 0.01 pituglandol, marked relaxation with diminished pendular movements occurred immediately. These effects were readily antagonized by adding an equivalent amount of calcium chloride.

In experiments with sodium citrate Sabbatani[15] succeeded in preventing coagulation of blood and lymph in vitro, as well as in the intact animal. The antagonistic action of calcium chloride and citrate was also demonstrated by this writer. Since calcium citrate is quite soluble he concluded, however, that its inhibiting effect on coagulation was due to the transformation of calcium into a non-ionized condition. He was led to the same conclusions by his experiments with rennin. The non-coagulability of sweet milk, he believed, may be explained by the presence of calcium in the form of the citrate. The action of sodium citrate on rennin and the antagonistic effect of calcium were corroborated

[12] Chiari and Froelich: Archiv für experimentelle Pathologie u. Pharmakologie, 1911, **64,** p. 214.

[13] Decottignie: Dissertation, Paris, 1901–1902.

[14] Sugimoto: Archiv für experimentelle Pathologie u. Pharmakologie, 1913, **74,** p. 27.

[15] Sabbatani, L.: Archives Italiennes de Biologie, 1901, **36,** p. 417.

later by Vietinghoff-Scheel.[16] Loeb,[17] experimenting with striated muscle, observed that it contracts powerfully or it may even go into tetanus when brought in contact with air, oil, or other indifferent solutions after previous immersion into solutions of sodium tartrate, oxalate or citrate. Since this phenomenon, which he termed contact irritability, may be inhibited by adding small amounts of calcium chloride to substances that are calcium precipitants, Loeb concluded that decrease of the calcium ions in the tissues is the cause of this peculiar action. He made, however, the plausible statement that these salts may have a specific effect upon other constituents of the protoplasm, such as protein. The calcium theory was also suggested in explanation of the action of the purgative salts. Loeb observed that salts which precipitate calcium are the most powerful stimuli of nerve and muscle and that they also exert a purgative effect. Their action would, therefore, be the same whatever the mode of administration, for contact with the cell is all that is necessary in order to produce the desired effect. Macallum[18, 19] and Bancroft[20] put this theory to an experimental test. Experiments with citrates and tartrates given by subcutaneous injection into rabbits produced purgation which could be inhibited by calcium chloride. Their results would seem to be contradicted, however, by the experiments of Auer,[21] as he failed to produce purgation in rabbits by the subcutaneous injection of sodium citrate. That the action of this salt is due to the formation of a compound with calcium was contended later by Robertson and Burnett.[22] According to these observers tolerance to sodium citrate may be produced in rabbits and is due to adaptation to a lower calcium level.

The calcium theory, it may be noticed, has gained adherents among many workers who sought an explanation for the pharma-

[16] Vietinghoff-Scheel: Archives internationales de pharmacodynamie et de therapie, 1902, **10**, p. 145.

[17] Loeb: American Journal of Physiology, 1901, **5**, p. 362.

[18] Macallum: University of California Publications, Physiology I, 1903, p. 5.

[19] Macallum: University of California Publications. Physiology II, 1905, p. 65.

[20] Bancroft: Journal of Biological Chemistry, 1907, **3**, p. 191.

[21] Auer, J.: American Journal of Physiology, 1906, **17**, p. 15.

[22] Robertson and Burnett: Journal of Pharmacology and Experimental Therapeutics, 1912, **3**, p. 635.

cological action of oxalates, citrates and tartrates. A notable exception to this view was taken, however, by Gros[23] in a recent communication on this subject. He found in experiments on the isolated frog heart that sodium citrate is decidedly more toxic than sodium oxalate, which is a fact of considerable importance for calcium citrate is quite soluble, a saturated solution at room temperature being about 1 gram per liter, while calcium oxalate is practically insoluble. The calcium precipitation theory is, therefore, untenable according to this observer.

In the course of investigations on the action of sodium tartrate on the circulation carried on in this laboratory it was observed that the effect of this salt may be promptly abolished by the intravenous injection of sufficient quantities of calcium chloride. The antagonistic action of the calcium and tartrate in the intact animal pointed therefore to disturbance of the calcium mechanism as the cause of the action of the tartrate ion. It seemed, however, that a more definite answer concerning this mechanism might be obtained by making a comparative study of the action of salts closely allied with respect to the solubility of their calcium compounds. This coupled with the obvious advantage to the solution of a problem gained by experimentation under conditions much less complicated led to the systematic study of sodium tartrate, citrate, and oxalate on the isolated heart.

METHODS

The isolated heart of the dog, cat and frog was used in these experiments. Perfusion of the mammalian heart was carried out as follows: The animal was anesthetized and bled from the carotid artery, Locke's solution being introduced at the same time into the femoral vein. We found that the amount of blood removed was an important factor in the success of the experiment. The heart of the animal that was bled until it was almost exsanguinated frequently failed to give satisfactory results. It soon became oedematous and after a short time it stopped in systole. Uniformly good results were obtained, however, when bleeding

[23] Gros: Archiv für experimentelle Pathologie u. Pharmakologie, 1913, **71**, p. 395.

was moderate or, better still, when the subject was not bled at all before removal of the heart. The Langendorff apparatus was used, a double walled glass chamber in which the heart was suspended being substituted for the original metallic chamber as recommended by the author of this method. It was raised above the bath and kept at the same temperature as the perfusing fluids by circulating water in the intervening space.

The pressure in the perfusion fluids and in the heart was carefully regulated, all experiments being carried out under uniform pressure. The same precaution was observed with regard to the temperature of the fluid as it entered the heart. Special tests, however, to ascertain the effect of temperature have shown that small differences do not materially influence heart action. Changes in pressure, on the contrary, have produced considerable differences in cardiac action.

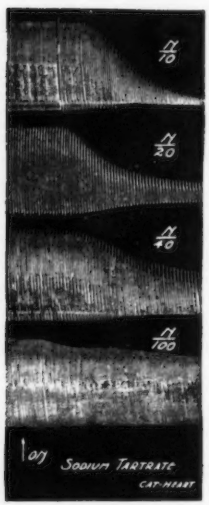
The frog's heart was perfused by means of a cannula which was introduced into the sinus venosus, the other veins being tied. The fluid thus passed through the heart, leaving it by way of the aorta. The solutions used for perfusion were contained in reservoirs which terminated at the lower end into glass tubes provided with stop cocks. By means of rubber tubing each reservoir was connected with the heart cannula. A free flow of the solutions was allowed, the perfusing fluids being thus replaced continually. The fluids in the reservoirs were kept at approximately constant pressure.

Experiments with Sodium Tartrate

Previous investigations on this subject have been made by Karczag,[24] who observed decreased cardiac activity and heart block when the isolated heart of the turtle was perfused with $\frac{N}{100}$ tartaric acid. Depressed heart action was also obtained by Gros[25] and by Sakai[26] in experiments with tartrates when the isolated heart of the frog was perfused.

[24] Karczag: Zeitschrift für Biologie, 1909, **53**, 218.
[25] Gros: l.c.
[26] Sakai: Zeitschrift für Biologie, 1914, **64**, p. 1.

Our own investigations with sodium tartrate, which were carried out on the heart of the dog, cat and frog, have likewise shown depressed heart action which varied, with the concentration of the solution used. Thus perfusion of the cat's heart with solutions of $\frac{N}{10}$, $\frac{N}{20}$, $\frac{N}{40}$ and $\frac{N}{100}$ sodium tartrate in defibrinated blood, diluted with Locke solution, or in Locke solution alone, was followed by diminished activity of the heart which became more marked as the concentration of sodium tartrate was in-



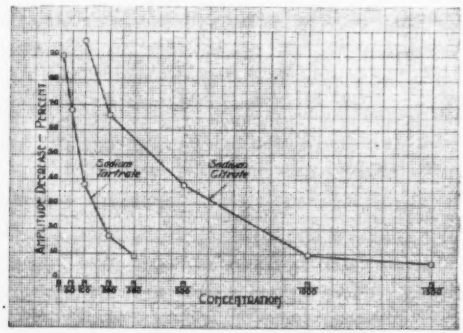


Fig. 2. Graphic comparison of effect on the frog-heart of equivalent quantities of sodium citrate and sodium tartrate dissolved in Ringer solution. Perfusion time in all cases 30 seconds, each point representing an average of at least two determinations. The difference between the smallest amplitude obtained on perfusion with a given concentration, and the amplitude just before perfusion, divided by the latter quantity gives the amplitude decrease in per cent.

Fig. 1. The response of the isolated cat-heart to four concentrations of sodium tartrate in Locke solution.

creased (fig. 1). It may be remarked, however, that the action did not vary in the same ratio as the concentration. The effect of various dilutions was even better exemplified in experiments on the frog's heart (fig. 3) in which very dilute solutions were employed. When $\frac{N}{300}$ sodium tartrate was perfused for 30 to 60 seconds a slight cardiac depression was observed, the systole

alone being decreased in some experiments, in others the decrease affected diastole as well as the systole. This may be regarded as the minimum concentration which can produce any effect since a $\frac{N}{400}$ solution proved to be without any action. Cardiac activity was markedly depressed under the influence of $\frac{N}{200}$ sodium tartrate, the effect on the systole being more pronounced than on the diastole. This was also observed with more concentrated solutions. Although the effects varied with the concen-

Fig. 4. Shows the effect of perfusion time on the toxicity of sodium citrate.

Fig. 3. Tracings showing the results of 30-second perfusions with various concentrations of sodium tartrate in Ringer solution. When the heart is not working very strongly only systole is affected ($\frac{N}{10}$) while both systole and diastole are affected when the contractions have a large amplitude. Compare this figure with figure 6 showing action of sodium citrate.

tration of the salt, their ratios, as was observed in experiments with the cat's heart, were likewise unequal. This is apparent on comparing the action of $\frac{N}{100}$, $\frac{N}{50}$ and $\frac{N}{10}$ solutions of sodium tartrate, the decrease in amplitude being respectively 40, 70 and 90 per cent (figs. 2 and 3).

A very noticeable difference in the action of sodium tartrate was also obtained by varying the perfusion time. $\frac{N}{10}$ sodium tartrate perfused for one minute produced a marked decrease in amplitude in the dog's heart. When the perfusion time was increased to $1\frac{3}{4}$ or more minutes, cardiac action was much weaker and also became irregular. The effect of time was also noticeable in experiments with shorter intervals, and as will be shown later, was especially striking in experiments with sodium citrate (fig. 4).

Experiments with Sodium Citrate

There is a noteworthy paucity in the literature, of studies on the action of citrates on the circulation. Vietinghoff-Scheel[27] reported some experiments on the effect on blood pressure while a short notice was recently published by Gros,[28] Clark[29] and later by Sakai[30] on its influence on the isolated frog heart. We are not aware of any other published communications on the subject. In the experiment to be reported sodium citrate, dissolved in Locke solution, was employed in various concentrations, the cat heart and that of the frog being used. The effect, a description of which follows, may be considered typical of the action of sodium citrate on the heart of the cat.

A solution of $\frac{N}{10}$ sodium citrate produced complete cessation of cardiac action, which occurred about 30 to 40 seconds after the solution was turned on (fig. 5). For two minutes after the citrate was discontinued, Locke solution being substituted in its place, the heart was at a standstill. Although considerable improvement took place, the recovery was incomplete and very slow. Eight minutes after perfusion the contractions were still weak, the amplitude being about one-sixth the size before perfusion with the citrate. It may be remarked, however, that the rhythm was regular at this time.

[27] Vietinghoff-Scheel: l.c.
[28] Gros: l.c.
[29] Clark: Journal of Physiology, 1913, **47**, p. 66.
[30] Sakai: l.c.

The effect of concentration was well shown in experiments with weaker solutions. $\frac{N}{20}$ sodium citrate (fig. 5) perfused for one minute was just sufficient to suspend heart action about the end of the period, but the contractions were just as vigorous one minute after Locke solution was turned on as before the citrate was perfused, the amplitude continuing to increase during the next 30 seconds until it became 30 per cent greater than it was during the fore period. The rate was only slightly accelerated. A number of experiments with $\frac{N}{40}$ solution was also carried out. In every case heart action was reduced considerably, the individual beats becoming very feeble in some instances, in others they were still fairly strong at the end of one minute perfusion with citrate. Heart action, however, was never inhibited

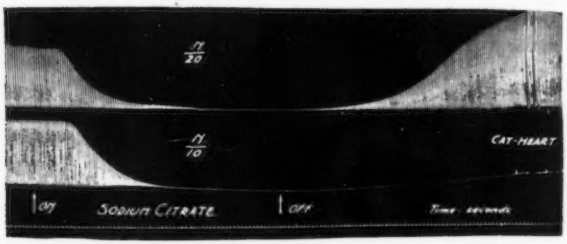


Fig. 5. Action of two concentrations of sodium citrate in Locke on the cat's heart. Cf. figure 1.

completely as was observed with stronger solutions. Except in one experiment the activity of the heart in the after period was greatly augmented, the force being appreciably greater on recovery, but, as was already stated under sodium tartrate, this lasted a brief period after which it became normal again. It is worthy of notice that rhythm was not appreciably affected during these experiments.

Experiments with citrate on the isolated frog heart have shown even more clearly the influence of concentration (figs. 2 and 6). It was found that a very dilute solution, $\frac{N}{1500}$, sodium citrate perfused for two minutes was still active though very slightly so but a solution of $\frac{N}{1000}$ perfused for 30 seconds to one minute showed a well marked decrease in amplitude with prompt

recovery when the perfusion with the citrate was discontinued and Ringer or Locke solution allowed to pass through the heart. Solutions of $\frac{N}{500}$, $\frac{N}{400}$, $\frac{N}{200}$ and $\frac{N}{100}$ were also tested. As shown in figure 6 the force of cardiac contraction diminished as the concentration was increased. The amplitude after perfusion with a $\frac{N}{100}$ solution for 30 seconds promptly diminished so that the heart beats were noticeable when magnified. Attention may be called here to the interesting phenomenon already observed

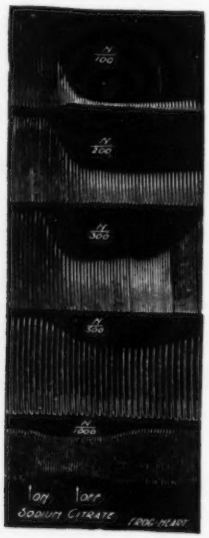


Fig. 6. Tracings of the effect of sodium citrate in Ringer on the frog-heart. Cf. the extent of the effects observed here with that in figure 3.

with sodium tartrate, namely, the augmentation of cardiac action in the after period. This was even more marked with sodium citrate, especially when concentrations above $\frac{N}{1000}$ were employed. In some experiments the amplitude was doubled when Ringer or Locke solution was perfused after previous treatment with citrate but the rhythm was not disturbed. There was never any irregularity such as was observed under similar

conditions in the cat's heart. The decrease in amplitude with different concentrations is best shown in figures 2 and 6. It will be noticed that the decrease is not proportional to the increase in concentration. Thus after perfusing with $\frac{N}{300}$, for example, the amplitude decreased 38 per cent; when the concentration was $\frac{N}{200}$ the decrease was about 65 per cent.

Attention may be called in this connection to the influence of the perfusion time. As is shown in figure 4 marked differences were observed. When perfused for 10 seconds depression was pronounced but the contractions were good at the end of the experiment, recovery being prompt. But when the same heart was perfused for 30 seconds, cardiac depression was greater and recovery was delayed.

The Comparative Action of Tartrate, Citrate, and Oxalate

If sodium tartrate and citrate be now compared it will be observed that in the same concentration the citrate is a more powerful depressant than the tartrate. This is shown with great clearness in experiments on the frog heart. Thus the amplitude, as shown in figures 2, 3 and 5, was decreased nearly 10 per cent with $\frac{N}{300}$ sodium tartrate, while the decrease with $\frac{N}{300}$ sodium citrate was 50 per cent. It may be remarked here that $\frac{N}{400}$ sodium tartrate had no effect on the frog heart while this concentration of sodium citrate decreased the amplitude approximately 45 per cent. The difference was about the same with $\frac{N}{200}$ solutions, while a solution of $\frac{N}{100}$ showed a decrease of 38 per cent with sodium tartrate and 95 per cent with sodium citrate. The results obtained with the heart of the dog and cat have similarly shown that sodium tartrate is much weaker than citrate. This is well illustrated in figures 1 and 5 in which the effects of $\frac{N}{10}$ and $\frac{N}{20}$ of the tartrate and citrate are shown. Similar results were obtained with $\frac{N}{40}$ solutions.

The comparative action of sodium citrate and sodium oxalate was also studied. Owing to the insolubility of calcium oxalate in neutral salt solutions a Locke solution, minus calcium, was used in which the salts to be tested were dissolved. An isotonic sodium chloride solution was also tried, but this was abandoned

as it was found unsatisfactory. Heart action became feeble and uncertain when perfused with this solution. Gros,[31] and more recently Arima,[32] reported that a sodium chloride solution alone may sustain the heart of the frog. Their method, however, was quite different from the one employed in the present investigation. They filled the heart with small quantities of fluid, 1 to $1\frac{1}{2}$ cc., which they allowed to remain for some time instead of continually changing the perfusing fluid as was done in our experiments. According to Arima the immediate effect of such a solu-

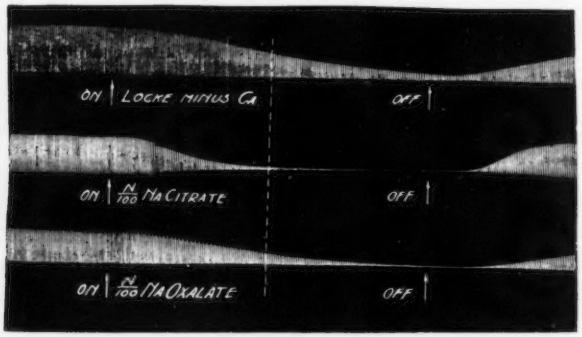


Fig. 7. Effect on the isolated cat-heart of sodium citrate and sodium oxalate dissolved in Locke solution minus calcium. The effect of a Locke no calcium solution on the same heart is shown for comparison. The perfusion time is two minutes, the broken line marking the close of one minute's perfusion. Citrate is seen to be the more toxic anion.

tion, even when small quantities were used, was to bring the heart to a standstill, from which it recovered, however, within ten to fifteen minutes.

Control experiments with calcium-free Ringer or Locke solution, which we carried out on the hearts of cats and frogs, indicated a decided decrease in cardiac activity, but the effect was much greater when such a solution containing either citrate or oxalate was perfused for the same length of time (see figs. 7, 8 and

[31] Gros: l.c.

[32] Arima: Archiv für die gesammte Physiologie des Menschen und der Thiere, 1914, **157**, p. 531.

9). In some experiments a moderate activity of the heart was still present at the end of two minutes' perfusion with a calcium-free solution, which was not the case when perfused with such a solution containing even moderate amounts of citrate or oxalate.

Comparative tests with citrate and oxalate were carried out with equi-molecular solutions of these salts, dissolved in Ringer

Fig. 8. Effect on the frog-heart of alternate perfusion with sodium citrate and sodium oxalate dissolved in Ringer minus calcium. Perfusion time is 10 seconds, there being two minutes between perfusions.

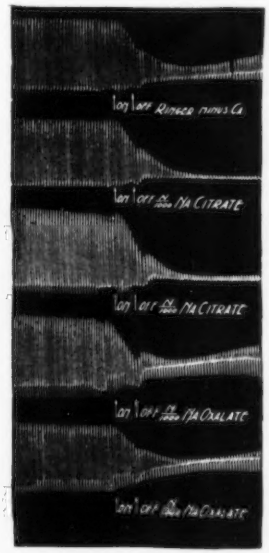


Fig. 9. Two consecutive perfusions with citrate, and two with oxalate give results on another frog-heart, again denonstrating the greater toxicity of citrate. The rate of perfusion in this heart was greater than that in figure 8.

or Locke—minus calcium. The results obtained indicate that in some cases oxalate has the same activity as citrate, but in a much larger number of experiments the citrate was decidedly stronger than the oxalate. This is particularly well illustrated in experiments on frogs when the perfusion time lasted only ten seconds

(figs. 8 and 9). When the perfusion time was longer the difference was much less marked. In experiments on the cat's heart similar results were obtained (fig. 7). A solution of $\frac{N}{100}$ (fig. 7) sodium citrate at the end of one minute's perfusion caused a considerable decrease in heart action, the amplitude as shown in the figure being markedly diminished. When $\frac{N}{100}$ sodium oxalate solution was perfused through the same heart four minutes later, the diminution of heart action at the end of one minute's perfusion was noticed to be much less marked. Recovery, however, was somewhat slower than after the citrate. A more concentrated solution has also shown that the citrate is more active than the oxalate. This appears very strikingly with perfusion for one minute of $\frac{N}{40}$ sodium citrate and $\frac{N}{40}$ sodium oxalate. Complete cessation of cardiac action for more than thirty seconds followed perfusion with citrate, while perfusion with oxalate of the same concentration seven minutes later caused a very marked decrease in amplitude, but the heart was contracting, though feebly, at the end of the period. Recovery in this case, too, was rather slow. The heart failed to attain the same strength as after the citrate.

DISCUSSION

As observed in the introductory remark, precipitation of calcium in the cell and tissues was offered as an explanation of the action of tartrate, oxalate, and citrate. The results we obtained we believe justify the conclusion that this theory is untenable, for if the precipitation of calcium in the tissues were the cause it would follow that the salts which form the most insoluble compound with this metal, would be the most toxic. According to this theory sodium oxalate ought to be the most toxic of the three salts under consideration, for its calcium salt as given by Richards, McCaffrey and Bisbee[33] is very slightly soluble in neutral solution, while sodium citrate ought to be the least active because calcium citrate is the most soluble of the three salts. Its solubility was found to be 0.0959 grams per 100 cc. according

[33] Richards, McCaffrey and Bisbee: Zeitschrift für anorganische Chemie, 1901, **28**, p. 85.

to Partheil and Hübner.[34] Sodium citrate ought, therefore, to be also less toxic than sodium tartrate for the solubility of calcium tartrate, as determined by Cantoni and Zachoder,[35] is 0.0525 per 100 cc., which is about half that of calcium citrate. Our experiments have unmistakably shown, however, that sodium citrate is the most toxic, while sodium tartrate is least toxic. The comparative strength of these three salts is illustrated in figures 2, 3, 8 and 9.

Transformation into non-ionized compounds was the other theory suggested to explain the action of these salts. Experiments undertaken to test this theory utterly failed to furnish any evidence of such a change. When the heart of the cat was perfused with solutions of calcium tartrate or citrate in various

Fig. 10. Locke-tartrate means a Locke solution in which the $CaCl_2$ was replaced by an equivalent quantity of Ca tartrate. The toxic effect of the small quantity of tartrate ion is completely masked by the effect of the Ca ion. Locke-citrate is a Locke solution in which $CaCl_2$ is replaced by an equivalent of Ca citrate. The greater toxic action of the citrate ion is shown by the inability of the Ca ion to completely mask its effect. The record was taken with the isolated cat-heart.

concentrations it was found that these salts could be as readily utilized as calcium chloride. Figures 10 and 11 show that when experiments were conducted with Ringer or Locke solution, calcium citrate or tartrate being used instead of calcium chloride, the same amount of calcium being contained in the solution in each case, the results obtained were the same. Little or no difference due to the tartrate or citrate ion could be observed.

[34] Partheil and Hübner: Archiv der Pharmazie, 1903, **241**, p. 413.

[35] Cantoni and Zachoder: Bulletin de la societé chimique de France, 1905, **33**, p. 747.

In this connection Clark's[36] observations are exceedingly interesting. He found that the insoluble calcium oleate formed by adding its soluble sodium salt to Ringer's solution can revive the isolated frog heart that has been fatigued by continuous perfusion with this fluid.

Attention may also be directed to the rapid action of the salts observed in our experiments in which perfusion for ten seconds only was sufficient to produce marked effects. No satisfactory explanation can be offered at present, but in the light of the investigations on the permeability of the cell to organic

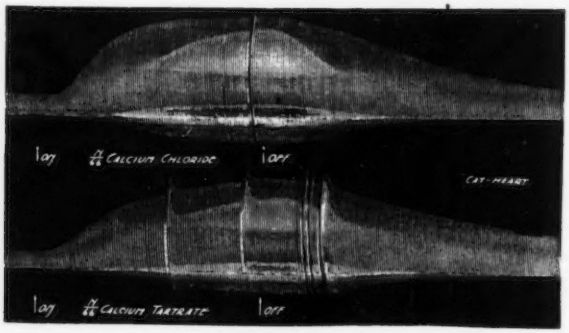


Fig. 11. Effect on the cat's-heart of equivalent quantities of $CaCl_2$ and Ca tartrate dissolved in Locke. This, as well as figure 10, shows that the toxicity of tartrate and citrate cannot be attributed to a transformation of calcium into compounds that the heart cannot "use."

acids and neutral salts, surface action suggests itself. According to recent observations of Harvey[37] the rate of diffusion of oxalic, tartaric and citric acid into the cell is twelve, fifteen, forty and thirty minutes, respectively, while Overton[38] previously demonstrated that organic salts penetrate into the cell even much more slowly than their acid. Harvey[39] has also shown that while the cell is permeable to some dyes in the acid condi-

[36] Clark: l.c.
[37] Harvey: Science, N. S., 1914, **39,** p. 947.
[38] Overton: Archiv für die gesammte Physiologie, 1902, **92,** p. 115.
[39] Harvey: J. Experimental Zoology, 1911, **10,** p. 507.

tion, it resists their extrance as a neutral salt. It has been suggested that the action of kations is also extracellular. Overton[40] claimed that potassium and calcium penetrate the striated muscle fiber very slightly if at all and maintained, therefore, that the action of electrolytes on striated muscle is confined to the surface. Boehm[41] more recently adopted the same explanation for the action of potassium and calcium on the isolated heart of the frog, for the rapid action of their salts would have been impossible if the effect observed depended upon their penetration into the muscle cell. The prompt recovery observed in our experiments when Ringer and Locke solution was perfused after the previous treatment with citrate, tartrate, or oxalate may also be regarded as additional evidence against internal changes produced by these salts. If any damage were done to the cell, as might indeed be expected if these substances penetrated into the interior of the muscle fiber, it is extremely doubtful whether the heart could resume its normal activity almost momentarily and not only make a perfect recovery but even exhibit for an appreciable length of time augmented activity without any untoward after effects. No definite statement can be made, however, as to the nature of the extracellular changes involved. Whether it is due to loss of lipoids, as suggested by Clark's[42] observations, or to some physico-chemical changes at the surface of the cell cannot be told at present for our knowledge of the factors concerned in the phenomena observed is as yet wholly inadequate.

SUMMARY AND CONCLUSIONS

1. The results obtained in this investigation show that sodium tartrate is much less active than citrate or oxalate.

2. Equi-molecular solutions of the latter perfused for the same length of time indicate that sodium citrate may be more toxic than oxalate.

[40] Overton: Archiv für die gesammte Physiologie, 1904, **105**, p. 176.

[41] Boehm: Archiv für experimentelle Pathologie und Pharmakologie, 1914, **75**, p. 230.

[42] Clark: l.c.

3. The action varies with the perfusion time, the longer the heart was perfused the greater the toxic effect.

4. The data obtained do not support the calcium precipitation theory as an explanation of the action of these salts since the effect seems to be independent of the solubility of their compounds with calcium. Our conclusions are therefore in harmony with those of Gros.

5. The theory that the action is due to the formation of non-ionized calcium salts is likewise untenable for calcium tartrate and citrate can be utilized by the heart.

6. The rapid action indicates that the changes produced are not due to penetration into the cell since, as was found by Overton and Harvey, diffusion of neutral salts into the cell takes place very slowly.

It is suggested, therefore, that the effects are due to extracellular changes.

AUTOLYSIS AND INVOLUTION

MAX MORSE

From the Department of Physiology, University of Wisconsin, Madison

Received for publication November 4, 1914

Our knowledge of *in vivo* autolysis rests upon comparatively few observations. Martin Jacoby[1] found free leucin and tyrosin in necrotic portions of ligated liver.

Green[2] on the botanical side, described autolytic changes in seed germination; other botanists[3] have likewise done this although Pond[4] failed to find the date-seed embryo capable of self-digestion. P. A. Levine[5] found evidences of autolysis in developing eggs of fish and fowl. The circumstantial evidence that the only adequate explanation of disuse atrophy and kindred phenomena is autolysis may complete our invoice of data upon autolysis in the body.

Three years ago, the present writer conceived the idea of studying the process of involution in the larval frog with special reference to the possibility of autolysis being the principal factor, for here is an ever-available supply of material. With the newer methods for studying small amounts of material (Folin's, Van Slyke's, Abderhalden's, Sörensen's, etc.), the problem would not seem difficult. In reviewing the literature, which is quite extensive,[6] it became evident that no work had been performed upon the physiology of the problem, all having been concerned

[1] Zeitschr. f. physiol. Chemie, 30, p. 149.

[2] Proc. Roy. Soc. London, 47, p. 147.

[3] Grüss: Ber. D. bot. Ges., 11, p. 288; 12, p. 60. Brown and Morris: Journ. Chem. Soc., 57, p. 458. Van Tieghem: Comptes rendus, 84, p. 582. Gris: Ann. Sci. Nat. Bot. (5) 2, p. 90. Hansteen: Flora, 79, p. 419. Linz: Jahrb. wiss. Bot., 29, p. 267.

[4] Annals of Botany, January, 1906.

[5] Zeitschr. f. physiol. Chemie, 35.

[6] Cf. Mercier: Arch. d. zool. expér. et gén. (4) 5, p. 1, for bibliography.

with histological aspects. Phagocytosis was accepted almost in every case as the efficient factor, dating from the classic investigations of Metschnikoff[7] on the absorbing tail of the tadpole of the frog, which became the foundation for his theory of phagocytosis. One, alone amongst the number of students of involution advocated any process other than phagocytosis (Looss).[8] While Looss had nothing other than histological data to present, yet he advocated the process of physiological absorption—"einer Auflösung, mit einer Resorption im strengen Sinne des Wortes."[9] The tail he likens to "der Dottersack für den sich entwickelnden Embryo" and like the yolk-sac, "die Dottermaterial wird ohne Hülfe von Leuko- oder Phagocyten aufgebraucht." For insects, during the period of metamorphosis, the exhaustive researches of Janet[10] show that "au cours de la dégénérescence, c'est-ā-dire depuis le moment ou le muscle est encore intact jusqu'à celui où il n'en reste plus d'autre trace que les enveloppes de ses faisceaux, il n'y a certainement aucune phagocytose c'est-à-dire aucun englobement, par un leucocyte amiboïde, de particules solides du muscle en histolyse."[11] The process is conceived as "une histolyse par digestion cavitaire due à l'action des diastases du sang lesquelles diastases, sans action sur le tissu vivant, apte à se défendre l'attaquent des qu'il est mort."[12] In this opinion, Janet is supported by Korotneff,[13] Miall and Hammon,[14] Karawaiew,[15] Rengel,[16] Berlese,[17] Terre,[18] et al.

[7] Biologisches Centralb., 3, p. 560.

[8] Looss: Preisschriften von der Fürstlich Jablonowski'schen Gesellschaft in Leipzig. XXVII. Verlag von B. S. Hirzel in Leipzig.

[9] L. c., p. 91.

[10] Anatomie du corselet, etc. Limoges: Ducourtieux et Gout (privately printed).

[11] P. 134.

[12] P. 133.

[13] Biol. Centralb., 12, p. 261.

[14] Trans. Linnean Soc. (2) 5, p. 265.

[15] Biol. Centralb., 19, p. 122.

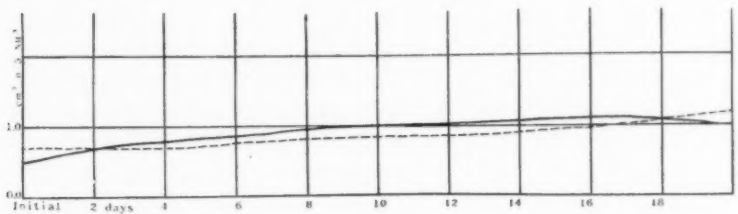
[16] Zeitschr. f. wiss. Zool., 62, p. 1.

[17] Zool. Anzeiger, 23, p. 441.

[18] Bull. Soc. entom. France, 1900, p. 62.

Atrophy, such as occurs in muscle degeneration in man (myositis, dermatomyositis, etc.) is nearly universally ascribed to a process involving phagocytes.[19] How much the atrophy of disuse is interpretable upon this theory, or upon any other is unknown at present. The similarity of histological pictures and descriptions of muscle degeneration and atrophy in the larval frog's tail is striking, lending a certain basis to the belief that fundamentally, there is a correspondingly similar physiological substratum; that is, that the pathological states described by Senator, Strümpel and others are reflected in the normal absorption of the tadpole's tail.

That phagocytosis is the efficient factor in the involution of the tail of the frog larva is improbable from the following considera-



Showing the relation between autolyzing muscle in a normal condition and when it has undergone partial degeneration after its innervation has been cut. Continuous line = control; broken line = experiment.

tions: In the first place, it is exceedingly doubtful that phagocytes affect normal tissue, so that we must logically assume a precedent lesion, something perhaps as Bataillon[20] has done in regard to the growth of the urostyle, which cuts off the blood supply, leading to an asphixiation of the tail tissues. Secondly, the rapidity of the process is scarcely coördinate with the activity of phagocytes; normally, over a half of a gram,[21] dry weight, of tissue becomes absorbed during two weeks, but while this is not especially striking, what of the anomaly when on feeding thyroid,

[19] Cf. Steiner: Journ. Exp. Med., vol. 6, 1904.

[20] Comptes Rendus (9), 2, p. 137.

[21] In Rana pipiens; in Rana catesbiana, this may be tripled.

which Gudernatsch[22] has shown to accelerate the process of absorption over two-thirds, so that even three days show the complete absorption of the tail tissues.[23] Again, histological studies reveal the relatively small number of phagocytes; that the process of atrophy begins at least in some tissues (epidermis, Looss), before the advent of phagocytes; that along with the phagocytes, erythrocytes appear which are to be accounted for in these locations only by rupture of the blood vessels since diapedesis does not occur in the red cells; this rupturing seems to indicate the previously weakened condition of the walls of the vessels and of the sarcolemma (Barfurth, Looss). In myositis, the degeneration of the muscle takes place centrifugally, not centripetally, so that the core of the muscle fibres and not the periphery, where the phagocytes occur, exhibits the degenerative changes first (Steiner). Thirdly, I have shown[24] that there is no concomitant rise in leucocytes and especially of the polynuclear variety, during the process of involution, a rise which should be expected if the process is due to the activities of phagocytes derived from the blood;[25] leucocytosis should appear here as in corresponding cases in mammals.

The various theories as to the nature of the impulse causing involution which have been offered, are inadequate. Barfurth[26] suggested disuse degeneration, but anyone familiar with tadpoles will bear witness to the fact that the tail is used during swimming movements even during metamorphosis. Bataillon's[27] "asphixiation theory," involving a cutting off of the blood supply

[22] Arch. f. Entwicklungsm., 35, 457 (1912).

[23] As to what the special factor in thyroid is, the present writer has shown (Journ. Biological Chemistry, 19, No. 3, 1914) that iodin in some association with thyroid globulin or even iodized amino-acids (3, 5, diodo-tyrosin) produce the same result as intact gland substance as used first by Gudernatsch.

[24] Proc. Soc. Exp. Biol. and Med., 10, p. 31.

[25] Although Metchnikoff first postulated that the phagocytes were not blood cells, but arose *in situ*, yet the researches of Mercier have shown that the cells which Metchnikoff described as phagocytes circulate in the blood stream and in histological sections, they are shown to be leucocytes.

[26] Also: Bohn, G.: Comptes rendus, 56, p. 661.

[27] Comptes rendus (9) 2, p. 137.

is not favored by my experiments in ligating the dorsal aorta of normal and of involuting larvae with the result that no effect upon metamorphosis in any case was visible. Barfurth's[28] further idea that hunger may be the "förderndes Prinzip" can scarcely be true, for Bábak showed in 1906 that larvae do feed and moreover, the studies of Gudernatsch, myself and others, where food is administered (thyroid) show that involution occurs when food is present. The corresponding case of the Rhine salmon described by Miescher,[29] where no food is taken during the atrophy of the muscles and the formation of gonads, is not a parallel one to the larval frog, nor is the instance of larval insects the same, for in both cases, masses of food are laid up previous to metamorphosis.[30] Loeb[31] has shown that the nervous system has no influence on metamorphosis and I can corroborate this observation by my experiments upon severing the nerve cord of normal and involuting individuals.

With regard to autolysis, it must be admitted that the case at present is not clear. I have shown in another place[32] that there is no quantitatively demonstrable difference in amino-acid content in normal and in involuting larval tails. The suggestion was there made that perhaps the case was similar to that of the amino-acid content of the blood, earlier investigations failing to reveal the presence of these compounds, while later by aid of the micro methods to which I have referred at the beginning of the present article, these Bausteine were recognized by Van Slyke, Folin and Abderhalden and quantitatively estimated, even although they were rapidly withdrawn from the blood stream. But in the present instance, exactly these methods have been used.

The purpose of the following pages is to present evidence that

[28] Arch. f. mikr. Anat., 29, p. 28.

[29] Histochemisches und physiologisches Arbeiten.

[30] Storage of food does take place, of course, in the frog, to a slight extent as in the fat body, so that the case is even stronger against Barfurth's idea as to inanition.

[31] Archiv f. Entwicklungsm., 4, p. 502.

[32] Proc. Soc. Exp. Biol. and Med., 12.

in vitro autolysis is not accelerated in absorbing tissue over non-absorbing (which will be designated the control), as might be postulated if autolysis were the efficient factor.

The method used was the aseptic one of Salkowski,[33] toluene and chloroform being used for inhibiting bacterial growth. The material was kept in a thermostat at 30° C; while this temperature may not be optimum for poikilothermous animals like frog larvae, yet inasmuch as autolysis proceeded in the controls, this degree of heat does not inhibit the enzyme action.

Experiment, June 22. Tails from about twenty metamorphosing frog-larvae (*Rana pipiens*) were cut from the body at their bases, dried upon filter paper from adhering moisture and blood, balanced against a similar set from non-involuting larvae, ground in sand, made up to 250 cm.³ distilled water, covered with toluol and placed in a thermostat at 37° C. Left five days, shaken daily. Kjeldahl determinations upon 25 cm.³ aliquots of the tannic acid filtrates gave, for 25 cm.³ portions:—

Control	Absorbing
1.0 cm.³ $\frac{N}{5}$ NH^3	1.0 cm.³ $\frac{N}{5}$ NH^3 [34]

Similar results were obtained from a ten day period sample, showing that *no acceleration of autolysis appears in the absorbing tissue over the control.*

The above experiment has been repeated upon larvae of *Rana catesbiana*, in Connecticut, with the same results.

Experiment, August 11. 0.40 gram wet weight of material from the tails from each of a set of normal and involuting individuals of *Rana pipiens* was placed in 50 cm.³ Erlenmeyer flasks with 3 cm.³ H_2O (making thus approximately 5 cm.³ cultures). 2.5 cm.³ aliquots were examined after twenty-four hours by Van Slyke's gasometric method for amino-acids, as follows:—

Control		Absorbing	
Initial	0.350	cm.³ nitrogen over H_2O	0.325
24 hr. aliquot	0.385	cm.³ nitrogen over H_2O	0.410

These figures are all within the error of observation in the present instance, showing, again, that *no acceleration of autolysis is evident in*

[33] Die Deutsche Klinik, 11, 1903.

[34] Calculations are left thus since only relative weights were used.

absorbing over control material.[35] Taken in connection with the former experiment, I think that the case warrants the generalization that there is no evidence for acceleration of autolysis in the larval frog's tail.

It is difficult to determine the nitrogen partition of excreta, which might afford some evidence in the present case. Owing to the fact that during metamorphosis, the intestine voids itself of faeces, which contain bacterial remains, along with portions of undigested plant material, bearing nitrogen, collection of the urine is attended with unsurmountable difficulties; collection of the urine itself, if possible, involves loss of nitrogen in the form of ammonia and urea would be decomposed also. However, I placed five individuals from each of the two divisions, viz., control and absorbing, in a larger beaker containing 100 cm.³ distilled water and after twenty-four hours made Kjeldahls on the filtered liquid:

(100 cm.³ aliquot)

Control		Absorbing
0.80 cm.³	$\frac{N}{5}$ HCl	1.10

Ninhydrin being negative after filtering, there is little reason to believe that this difference concerns amino-acids. Moreover, urea determinations show that it is not urea nitrogen. In all probability, the result concerns defecated matter. During the time of this experiment, the weight changes were:—Beginning, 3.04 g. twenty-four hours, 2.44 g., average of five individuals; difference, 0.60 g. = loss in weight for twenty-four hours.

That the creatin nor creatinin component is altered, I have shown in another place[36] and while this criterion of muscle atrophy is held in question by some[37] as a valid index of endogenous metabolism, yet the point is of interest here.

It is impossible, therefore, with our present knowledge, to state whether involution in the larval frog involves a closed system, that is, that the materials from the tail act as "Dottermaterial" for the developing embryo as Looss suggested, and as

[35] The difference of 0.019 mmg. amino-nitrogen of the control and of 0.046 mmg. in the case of the absorbing tissue can scarcely be made a satisfactory basis for ascribing acceleration.

[36] Biochem. Bull., 1913.

[37] Mellanby: Journ. Physiol., 36, p. 447.

Miescher[38] and Noël Paton[39] found for the fishes developing gonads at the expense of other tissues.

Anglas,[40] a student of insect metamorphosis, has suggested that phagocytes may act "lyocytically," that is, excrete an enzyme which may attack the tissues, causing atrophy. This suggestion is similar to the process of heterolysis of Jacoby; but in our present case, inasmuch as heterolysis and autolysis would give similar results, we find no evidence for this view.[41]

Summarizing, we may say that no evidence is deducible from the foregoing experiments tending to show that autolysis is accelerated in the atrophying tissues of the larval frog.[42]

[38] (L.c.)

[39] Paton, Noël: Fisheries Board of Scotland, 1898, p. 175.

[40] Comptes rendus, Paris (11), 51, p. 947; 52, p. 219; Bull. Sci. Fr. et Belg., 34, p. 363.

[41] In one regard, the process of lyositosis is different from heterolysis, for the former method implies that the leucocytes are alive, while heterolysis may occur *in vitro*, after the tissues are dead. Note the relation to excretion of leucoprotease.

[42] That the absorption of tissues which have suffered lesions does not involve acceleration of tissue enzyme action is substantiated by the graph shown above, where the rates of autolysis in muscles (1) normal and (2) with nerve cut for one week, are compared. It will be noted that the control and experiment proceed in a similar manner. The method used was as follows: Rabbit, etherized, sciatic cut in one leg; asepsis throughout; wound sewn up, iodine, bandage; after one week, muscles concerned were excised, weighed wet, ground in sand, made to given amount of distilled water and allowed to remain in thermostat at 40° C. for twenty days, tannic non-precipitable nitrogen being determined by Kjeldahl method at two day intervals. Results in cc. $\frac{N}{5}$ NH_3.

CONTRIBUTIONS TO THE PHYSIOLOGY OF THE STOMACH

XVIII. On the Sensibility of the Gastric Mucosa

A. J. CARLSON and L. H. BRAAFLADT

The Hull Physiological Laboratory of the University of Chicago

Received for publication November 14, 1914

I. THE ABSENCE OF PAIN AND TACTILE SENSIBILITY IN THE NORMAL GASTRIC MUCOSA

Everyday experience tells us that the stomach mucosa is not sensitive to touch. Pawlow (15) states: "It can hardly be doubted that under normal conditions the surface of the stomach has a certain degree of tactile sensibility" (p. 90). The term tactile sensibility is evidently used here in the sense of a general response to mechanical stimulation rather than as implying a true tactile sensibility. When solid food is swallowed, no tactile sensation is felt after the food has passed the pharynx, unless the mass is so large that it causes unusual distension of the esophagus. In that case it may be felt all the way down the esophagus, but this is due to the distension of the wall of the esophagus and not a tactile sensation from contact with the mucosa.

Hertz (1) tested on himself the tactile sensibility of the mucosa of the esophagus by means of an esophagoscope with a slit down the side so that a metal bulb, in a long holder, could be moved along the mucous membrane of the esophagus and the pharynx. The pharyngeal mucosa was found to be sensitive to touch, the esophageal mucosa was insensitive

One of us (A. J. C.) tested the tactile sensibility of his own gastric mucosa, swallowing a good sized rubber rube, through which was passed a test tube brush attached to a strong piano

wire. Pulling the test tube brush about in the cavity of the stomach produced no sensation.

Numerous tests were made on the gastric mucosa of Mr. V., our gastric fistula case. Gently touching or striking the mucosa with blunt objects produces no sensation. If the mucosa is rubbed or pressed very vigorously with a blunt object Mr. V. says he "can feel it," he cannot describe the sensation thus produced except in a negative way. It is not like touch, nor is it pain or hunger (2). Whatever the character of the sensation may be we are satisfied that it is a real change in the stream of consciousness, for he recognizes these stimuli when he has no other means of knowing that the gastric mucosa is being handled. The sensation may not originate in the mucosa, but in the muscularis (tonus relaxation through reflex inhibition) or possibly in the visceral peritoneum, as the pressure must be considerable to produce it.

We are satisfied that the stimulation of the normal gastric mucosa of Mr. V. does not produce tactile sensation. The same conclusion has been reached by previous workers using human gastric fistula cases.

Gastric pain is a familiar clinical symptom, the hunger pangs, which are of gastric origin, are in reality hunger pains (2), and most of our readers have probably experienced the gastric pain produced by swallowing excessively hot food. Do any of these pains originate from stimulation of nerve endings in the gastric mucosa? We are satisfied from numerous tests on Mr. V. that *the sensation of pain cannot be produced from the normal gastric mucosa by any stimulation confined to the mucosa itself.* Pinpricks or incisions of the normal mucosa do not seem to affect consciousness in any way. It does not follow that pain may not be caused by the stimulation of the hypersensitive mucosa. We have no personal observations touching this point, but the literature seems to show that the gastric pain accompanying excessive inflation, gastric ulcers, and chronic obstruction is due to the mechanical stimulation of hypersensitive nerves or nerve endings in the muscularis or sub-mucosa by excessive distension or contraction.

II. THE SENSATION OF TEMPERATURE

In 1846 Weber suggested that the sensation of cold or warmth in the epigastrium, after drinking ice cold or very warm water, originates in the skin of the abdomen over the stomach and not in the stomach mucosa (3). According to Weber sufficient conduction takes place through the walls of the stomach and abdomen to stimulate the temperature nerves of the skin. Becher (4) swallowed a single rubber tube and through it injected water of different temperatures into the stomach. He did not experience any heat or cold sensation before the heat or cold has passed through the walls of the tube and stimulated the mucosa of the esophagus. Mueller concluded that ice water produced no sensation in the stomach. Zimmerman (5) irrigated his stomach with hot or cold water through a thick rubber tube. He claims that hot or cold sensations were felt only when the lower end of the tube was 30 to 35 cm. distant—hence not far from the lower end of the esophagus. He therefore concludes that the sensation is projected from the esophageal mucosa. Mackenzie (6) believes that the temperature sensations induced by hot and cold water into the stomach is due to reflex vaso-motor changes in the skin of the abdomen. Nystrom (7) reports that touching the gastric mucosa of a man with gastric fistula, with a hot piece of metal or piece of ice, does not call forth any definite temperature sensations. According to Hertz (1) the heat or cold sensations felt in the epigastrium, upon swallowing hot or cold water, comes from the lower end of the esophagus. The water accumulates here before the cardiac orifice opens and thus that part of the esophagus is stimulated more than the parts above. He warrants his conclusion by this observation: by auscultation over the epigastrium after swallowing a mouthful of very hot or cold water, one will find that immediately after the second deglutition sound, which occurs after the last trace of food has entered the stomach, the hot or cold sensation disappears. He also states that upon injecting hot or cold water through a double India rubber tube, no temperature sensation was noticed before three or four ounces had passed into the stomach. An ill-defined

temperature sensation was then experienced, but this was due to the conduction of heat or cold to the esophageal wall. He therefore concludes that the stomach mucosa is not endowed with heat or cold nerve endings. Quincke (8) introduced hot and cold water into the stomach of a boy with a gastric fistula and reports that the patient experienced vague heat or cold sensations. Neumann (9) and Roux (10) injecting hot and cold water into the stomach through a double rubber tube, experienced heat and cold sensations in the stomach, the cold sensation being the more pronounced. Head, Rivers and Sherren (11) injected water at different temperatures into the colon of a patient, upon whom a colostomy had been performed, and found that water at 20 to 40°C. produced no sensation at all but water at 50°C. and very cold water did give rise to temperature sensations, but that these sensations were by no means as pronounced and as easily localized, as those produced by applying water at the same temperatures to the skin. Head concludes that the viscera is endowed with protopathic temperature sensibility only.

It is evident from the above that by far the majority of those who have investigated this problem, have experienced a vague heat or cold sensation, in the region of the epigastrium, upon stimulating the stomach mucosa with very hot or very cold water. Those who do not believe that the sensation arises in the stomach mucosa explains its origin in one of the following ways:

(*a*) It is due to conduction to the skin of the abdomen.

(*b*) It is due to reflex vascular changes in the skin of the abdomen.

(*c*) It is projected from the esophagus.

(*d*) It arises in the lower part of the esophagus.

Our own experiments on Mr. V. and on ourselves go to show that the above mentioned theories are not tenable. That is to say, that the gastric mucosa is endowed with protopathic temperature sensibility.

The first series of experiments was on Mr. V. He was blindfolded and was not told the nature of the experiments. The water 40–60 cc. was injected through a small rubber tube which was passed through the large permanent tube of the fistula.

With water at 50°C., he said that he felt a heat sensation in the stomach after a latent period of about five seconds. When the same amount of water at 10°C. was injected a cold sensation was felt after a slightly shorter latent period. A metal rod 5 mm. in diameter heated to about 50°C. was passed down through the fistula catheter until the end touched the mucosa of the dorsal wall of the stomach. A longer latent period elapsed here before any heat sensation was noticed. When, however, a small piece of ice, held by a pair of forceps, was brought into contact with the stomach mucosa, the latent period was not much longer than when the cold water had been injected. A cold sensation was distinctly felt. These experiments were repeated over and over again and Mr. V. was invariably able to recognize whether the mucosa was being stimulated with hot or cold media, provided the hot media was 45 to 55°C. and the cold at least 13°C.

The temperature sensations initiated by touching the gastric mucosa directly with hot or cold solids cannot come from the stimulation of the esophageal mucosa. The latent period is too short to allow temperature conduction to the skin of the abdomen. But since it is possible that the cardia and the lower end of the esophagus of Mr. V. are patent, water injected through the fistula may reach the lower end of the esophagus. This possibility was guarded against in a second series of experiments, where we introduced the hot and cold water into a very delicate rubber balloon (condom) previously introduced into the stomach. The hot and cold water was correctly recognized as hot or cold, just as in the test with the water touching the mucosa directly, the only difference being a somewhat longer latent period. It is clear from the above that the gastric mucosa of Mr. V. is endowed with protopathic temperature sense.

A third series of experiments were carried out on ourselves. Three rubber tubes of suitable size were placed one inside the other so as to have three walls of rubber and two air spaces between the liquid in the inside tube and the esophageal mucosa, for the purpose of retarding heat conduction. The inside tube had a diameter of 3 mm. The subject was blindfolded and

50 cc. of water at 50°C. was injected into the stomach. A heat sensation was noted about ten to fifteen seconds after the injection, and about 60 seconds before enough conduction had taken place to cause the tube to feel warm in the mouth and throat. When the same amount of water at 10°C. was injected, a quite definite cold sensation was felt from five to ten seconds after the injection had commenced and about 60 seconds before the tube began to feel cold to the fingers or to the throat.

Both of us experienced heat and cold sensations and never made the mistake of confusing the heat sensation with the cold sensation. Confirming Neumann and others, we found the cold sensation to be more distinct and better localized than the heat sensation.

This certainly does not seem to indicate that the stomach mucosa is devoid of temperature sensations, nor that this sensation is projected from the esophagus, as has been suggested by a number of investigators. However, there is a possibility that some of the water might reach the wall of the lower end of the esophagus by being forced up along the tube. In view of the small amount of water necessary to evoke the heat and cold sensation and in view of the short latent period mentioned above, this seems very unlikely.

To make sure that the sensation did not arise as a result of stimulation of the esophagus, as well as to prove that the sensation is not due to conduction through to the skin of the abdomen, or to reflex vascular changes in the skin of the abdomen, a fourth series of experiments was performed. For these experiments an India rubber tube 1 cm. in diameter was used. Inside this large tube we placed two smaller tubes side by side; one of these was 5 mm. in diameter and the other was 3 mm. The smaller of these two tubes was not pulled clear through the outside tube, but only so far that its lower end was about an inch above the lower end of the outside tube. The 5 mm. tube was pulled through the outside tube so that it extended about 10 cm. below the lower end of it. A small glass tube 5 mm. long was now forced up into this longer tube as far as to the lower

end of the outside tube. The lower end of the outside tube was then tied securely about the longest tube at this point. This apparatus was now swallowed far enough down, so that the lower end of the outside tube reached just through the cardiac orifice. The longest tube, the end of which of course was open, therefore extended far down into the stomach. The smallest tube, whose lower end was within an inch of where the outside tube was tied, that is, within 2–3 cm. of the cardiac, was now connected with a pressure bottle. It had previously been found that hot water in passing through a glass tubing and connections of the pressure bottle lost approximately 8°C., and that water placed in the bottle at 10° was raised to 12°C. in passing through. Water at 58°C. (1) was now placed in the pressure bottle and permitted to flow down through the smallest inside tube. It, naturally, was forced up again between and around the two inside tubes. By so doing the heat was conducted through the wall of the outside tube and stimulated the heat nerve endings in the esophagus, so that a definite heat sensation was felt along its whole course, as well as in the mouth and pharynx. The striking feature about this, however, was that, although the walls of the lower end of the outside tube naturally became hot before those of the upper, the heat sensation was first felt in the mouth and throat and then gradually travelled down the whole length of the esophagus. At all times, however, the heat sensation was more intense in the throat and the upper part of the esophagus than in its lower end. While the walls of the esophagus were thus being stimulated, 50 cc. of water at 50°–55°C. was injected into the stomach through the longest inside tube which opened into the stomach and, after a latent period of about ten seconds, a spreading heat sensation was felt lower down than that resulting from the stimulation of the esophagus. The sensation also seemed better localized than that from the esophagus so that it was not very difficult to keep the two sensations apart in consciousness. The reason for the better localization might, of course, be due to the fact that more nerve endings were stimulated in the stomach mucosa, as the water came into direct con-

tact with it than was the case in the esophagus, where probably the rubber tube did not come into contact with the mucosa at all points.

When water at 10° was placed in the pressure bottle—the outside tube stimulating the esophagus thus being irrigated by water at 12°—and 50 cc. of water at 12° was injected into the stomach, a quite distinct, spreading, cold sensation was felt lower than that resulting from the stimulation of the esophagus. There was no difficulty in recognizing two separate and distinct cold sensations, one coming from the esophagus and one from the stomach.

While the esophagus was being stimulated by water at 12°C. in this tube system, a water bottle which was filled with water at 12°C. was placed on the skin of the abdomen. The two cold sensations resulting could easily be kept apart in consciousness. 50 cc. of water at the same temperature was then injected into the stomach and a third distinct, spreading, cold sensation was felt in the stomach. This experiment was repeated several times with hot and cold water as indicated above and with the same results.

If the temperature sensation felt, upon stimulating the stomach mucosa, is due to conduction through to the skin of the abdomen, or to reflex vascular changes in the skin of the abdomen, or is projected from the esophagus, how is it possible that one can distinguish in consciousness the sensations coming from the esophagus, the stomach, and the skin, at the same time, when each is being stimulated by water at the same temperature, as shown above?

To make sure that the sensation was not due to conduction through to the skin of the abdomen, the bulb of a thermometer was pressed close to the skin over the stomach and covered with absorbent cotton. After it had become stable, 200 cc. of water at 10° was injected into the stomach and the mercury in the thermometer watched for a period of five minutes. This was repeated several times. In each case, after a latent period of five minutes, the mercury had fallen only a small fraction of a degree. It is therefore evident that the cold sensation which one feels

after a latent period of from five to ten seconds, after the injection of 20 cc. of cold water into the stomach, is not due to conduction through the abdominal wall and stimulation of the nerve endings in the skin.

We agree with Hertz that when one swallows a mouthful of ice water, the most intense cold sensation seems to come from the lower end of the esophagus, and that this is undoubtedly due to the fact that the water accumulates there and is detained for a shorter or longer period before the cardiac orifice opens so that it can flow into the stomach. He further states that the cold sensation disappears after the water has entered the stomach. This we cannot corroborate. We invariably feel a vague spreading cold sensation after the water has entered the stomach, but which disappears after a few seconds. Even if it were the case that no cold sensation could be felt after the water had passed into the stomach, this could not prove that the stomach mucosa is devoid of heat and cold nerve endings. In the first place, a mouthful of cold water in passing down into the stomach, is warmed sufficiently to raise its temperature several degrees. On this account the nerve endings in the mucosa of the stomach are not stimulated as intensely as are those in the esophagus. The empty stomach contains 10–50 cc. of fluid at 38°C. which rapidly mixes with and thus raises the temperature of the swallowed water. It is also well known that stronger impulses, reaching the central nervous system from one part, tend to suppress in consciousness weaker impulses of the same nature, reaching it from some other part, so that it is difficult to separate the two sensations in consciousness.

From our experiments we conclude:

(*a*) The stomach mucosa is endowed with heat and cold nerve endings.

(*b*) These fibers are, as Head suggests, of the protopathic type.

(*c*) They are more abundant, or more readily stimulated in the throat and the esophagus than in the stomach.

III. THE GASTRIC COMPONENT OF THE SENSATION OF APPETITE

New facts touching the relation of the sensations of hunger and appetite, and particularly the peripheral sensory apparatus for hunger, have been reported from this laboratory in a previous paper (2). It was shown that the hunger sensation cannot be produced by any kind of stimulation of the nerve endings in the gastric mucosa. In the normal empty stomach the hunger sensation is initiated by strong contractions of the stomach wall, and by these contractions only. It was shown that these contractions, no matter how they are initiated, give rise to the characteristic sensations of hunger pangs. It was therefore concluded that the gastric sensory apparatus for the hunger sense lies in the muscular coats or in the connective tissue, but not in the mucosa. The hunger sense is a complex of kinestetic sensation (tension) and pain. Continued investigations on Mr. V., on ourselves, and on a number of other persons here in our laboratory have served to confirm the above conclusion. When an individual can clearly recognize the pangs of hunger he also recognizes (in suitable experiments) that these sensations are not induced by stimulation of the gastric mucosa.

Pawlow (15) concludes that "the tactile sensation of the stomach at the moment of entry of food is capable of awakening or increasing appetite" (p. 91). In the experiments cited by Pawlow in support of this conclusion the stimulation of the nerve endings in the mouth and in the esophagus was not excluded. However, the theory that stimulation of the normal gastric mucosa contributes to the sensation of appetite is clearly established by the experiments reported below.

One of us (A. J. C.) working on himself soon recognized that stimulation of the nerve endings in the gastric mucosa modified the flow of consciousness, although this modification did not consist in the sensation of hunger. This can readily be experienced by anyone who is sufficiently interested to try, by introducing moderately cold water, beer, wine, weak acids (0.5 per cent) or weak alcohol through a tube into the stomach so as to avoid stimulation of nerve endings in the mouth and esophagus.

The sensation produced by these substances in the stomach is rather transitory, but may persist for several minutes. With the exception of cold water which is also felt as cold, these various substances give rise to a characteristic sensation which fuses with, or cannot be distinguished from appetite. It is like the sensation of increased appetite experienced by most people at the beginning of a meal after eating a few morsels of palatable food. The sensation is pleasant, and invariably turns the attention towards food and eating.

We are accustomed to think that the above substances affect consciousness solely through stimulation of nerve endings in the mouth. This view is no longer tenable. By introducing these substances through the stomach tube at the height of a gastric hunger contraction one actually experiences a *successive contrast of the sensations of hunger and appetite*, as these substances temporarily inhibit the hunger contractions in stimulating the gastric mucosa. This method was demonstrated before the Federation of American Biological Societies in December last year (12). The experiment has been repeated a number of times on the junior author. From the first it was clear that, when beer, cold or hot water, were introduced into the stomach during a vigorous hunger contraction, the sensation resulting was the exact opposite of that caused by the hunger contraction. In place of an unpleasant, tense sensation, associated with restlessness, the sensation caused by these different stimuli is one of relief. A pleasant tingling sensation is felt in the stomach. One feels perfectly at ease, but the thoughts tend to revert to the dinner table. At first he was not able to say just what this sensation was like, although it was a familiar one. After paying close attention to the sensation experienced at meals just after a few mouthfuls of good food or drink have been swallowed, he became convinced that the two sensations are very much alike if not identical.

How do we know that the sensation temporarily produced by the above substances in the stomach is directly due to stimulation of nerve endings in the gastric mucosa? It has already been shown that the introduction of these substances in the

stomach temporarily inhibit the gastric tonus and the gastric hunger contractions (13). May not the sensation be one of negative character, so to speak, that is, diminution or absence of hunger? We are in position to answer this question definitely in the negative. In the first place, the sudden and spontaneous relaxation of the stomach at the end of a period of gastric hunger contractions is accompanied by a characteristic sense of relief and disappearance of a certain tension or unpleasant mental stress, but this sensation complex has not the positive character that directs attention to food and eating. It is essentially relief from pain. Secondly, putting these substances directly into the stomach which is quiescent and very greatly relaxed, still inaugurates this temporary appetite or appetite-like sensation. Hence we conclude that it is directly induced by stimulation of certain nerve endings in the gastric mucosa itself. Of course, if the nerve endings in the gastric mucosa are thus stimulated at the time the muscularis is in strong tonus and hunger contractions, the appetite-like sensation is fused with that of relief from the pangs of hunger.

It is significant that normal human gastric juice having full acid strength (0.45–0.50 per cent free HCl) is capable of inducing this sensation from the stomach. This has been verified repeatedly on one of us (A. J. C.) by introducing 50 cc. of appetite gastric juice of Mr. V. through the stomach tube. Gastric juice of weaker acidity (0.20 per cent) does not have this effect. As this full strength gastric juice is rapidly secreted into the stomach at the beginning of eating, it is probably a factor in the augmentation of appetite by the very act of eating. It may be pointed out that the above facts permit us to see a suggestion of truth in Beaumont's theory that turgescence of the gastric glands is the cause of hunger. In the first place, Beaumont, in common with most physiologists, did not clearly distinguish between hunger and appetite but used the two terms interchangably. If we, then, substitute appetite for hunger, we see that with the stomach normal the appetite sensation may be actually initiated or augmented by gastric juice, not through me-

chanical pressure of the juice in the ducts, but by acid stimulation of nerve endings in the gastric mucosa.

It need scarcely be pointed out that when foods or liquids are taken into the mouth and swallowed in the normal way their main influence on appetite is via nerve endings in the mouth. In fact, the latter is so predominant that only by excluding it are we able to clearly distinguish the gastric factor. The memory factor in appetite is therefore preëminently gustatory and olfactory.

We have as yet no device for recording objectively the *quality* of a *sensation*. In the preceding pages we have detailed certain conditions under which certain sensations are produced. We have called these sensations hunger and appetite, respectively, in accordance with our best efforts of subjective analysis. The experimental conditions are so simple that any biologist with a little perseverance can duplicate them. We believe they will verify our conclusions that

1. Hunger and appetite are qualitatively different sensations.
2. The sensory apparatus for hunger is distributed in the stomach wall and is stimulated by a certain type of contractions of the empty stomach.
3. The gastric mucosa is endowed with a protopathic appetite sensibility.

IV. THE SENSATION OF FULNESS

All who have studied the origin of the sensation of fulness seem to agree that it does not originate in the mucosa of the stomach, and our own results agree with this view. The literature is extensively reviewed by Hertz (1).

Hertz and his co-workers inflated the stomach of two healthy men with air through a tube connected with a manometer. They found that a "sensation of fulness or tightness in the upper part of the abdomen associated with a desire to eructate, was felt as soon as the intragastric pressure reached respectively 12 and 14 mm. of mercury in the two cases. The pressure fell after 20 seconds by approximately 2 mm., owing apparently to relaxa-

tion of the tone of the stomach, and simultaneously the sensation of fulness disappeared. Now on slowly injecting more air, the pressure gradually arose to its original height and the sensation reappeared; it again disappeared after 20 seconds, the pressure simultaneously falling 2 mm., after which it remained constant. Exactly the same rise in pressure and the same sensation of fulness, followed by a fall in pressure and a disappearance of the sensation, were produced four times in succession by injecting air, none being allowed to escape in the interval. These observations proved that the tension exerted from within on the circular muscle fibers of the stomach is the cause of the sensation of fulness."

That this is the main source of its origin seems quite clear. There is a possibility that the stretching of the abdominal muscles as well as the pressure on other structures in the abdominal cavity contribute to the sensation of fulness. As a proof that this element has little if any significance in giving rise to the sensation, Hertz related experiments on persons with atonic stomachs. When six grams each of sodium bicarbonate and tartaric acid are swallowed separately 1700 cc. of carbon dioxide are given off under atmospheric pressure at body temperature. This invariably causes an unpleasant and sometimes painful sensation of fulness in normal individuals while in persons with atonic stomachs it does not cause any sensation of fulness. Hertz therefore concludes that "the sensation of fulness in the stomach is due to tension on its muscular coat, and depends very little and only in extreme cases on the stretching of the abdominal wall."

To these observations of Hertz we can add our negative findings in regard to the gastric mucosa as a contributing factor. Chemical or mechanical stimulation of the mucosa (whether the stomach is in strong or in feeble tonus) never produces a sensation similar to that of fulness. At the same time, it must be noted that mere tension on the muscular coats, that is, intragastric pressure, will not under all conditions give rise to the sensation. The degree of intragastric pressure required to cause a feeling of fulness according to Hertz, is frequently ex-

ceeded at the height of a period of hunger contractions of the empty stomach, when a distended balloon is in the stomach; yet, the sensation referred to the epigastrium in these conditions is that of emptiness, not fulness. It is therefore clear that a *certain amount of tonus relaxation of the stomach must be present before tension or pressure on the walls of the stomach produce the sensation of fulness.*

V. THE EFFECT OF STIMULATING THE GASTRIC MUCOSA IN MAN ON THE VASO-MOTOR CENTER AND THE REFLEX EXCITABILITY OF THE SPINAL CORD

Effect on the Vaso-motor Center

Two series of experiments were performed (on L. H. B.), the first series with the subject awake and the second during sleep. The same method of experimentation was followed as that used by one of us in the experimentation on Mr. V. to determine the effect of the hunger contractions on the activity of the vaso-motor center (13). The man experimented on was blindfolded and endeavored to relax as completely as possib e.

In the first series, when relaxation was most comp ete, the same rhythm of the vaso-motor tone was noted synchronously with the strong hunger contractions as were observed on Mr. V., namely, an increase in the size of the arm during the strong gastric contraction. It was rather difficult, however, to become so completely relaxed that this phenomena could be seen.

When cold or hot water was introduced into the stomach through the tubes in quantities of 50–100 cc. at a time there was nearly always a shrinking of the arm, that is, vaso-constriction (fig. 1). Water at body temperature did not seem to affect the tonus of the vaso-motor center. Water at 10°C. caused less vaso-constriction than water at 55°C.

In order to ascertain whether this reflex was due to the influence on the vaso-motor center by conscious processes, similar experiments were performed while the subject was asleep. It is not an easy matter to go to sleep with a plethysmograph on one arm and two rubber tubes in one's throat and esophagus. But,

after a walk of about twenty miles followed by a long swim, it was possible. The same rhythmic variation in the vaso-motor tone was now observed as was seen while awake but perfectly relaxed. There was a gradual increase in the size of the arm as the tonus of the stomach increased. This increase or vaso-dilation was greatest when the stomach contraction was at its highest and the size of the arm decreased as the stomach relaxed. From this it seems evident that this activity of the vaso-motor center is not due to conscious processes.

Moderately hot or moderately cold water in the stomach did not seem to affect the activity of the vaso-motor center during

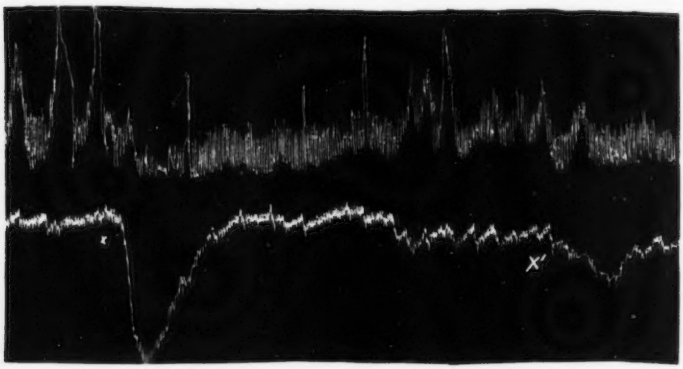


Fig. 1. Upper tracing from the empty stomach of L. H. B.; lower tracing from arm plethysmograph. *X*, introduction of 100 cc. water at 50°C. through double stomach tube into stomach; *x'*, introduction of 100 cc. water at 5°C. in the same way into the stomach. Showing reflex vaso-constriction from temperature stimulation of the gastric mucosa.

sleep. Very cold water either did not have any marked effect, or else the stimulation was sufficient to awake the subject. Warm water did not cause a marked vaso-constriction unless the subject woke up as a result of the injection. The marked vaso-constriction observed when the stomach mucosa is stimulated with very cold or very warm water therefore seems to be due mainly to the effect of conscious processes on the vaso-motor center.

Effect on the Reflex Excitability of the Spinal Cord

The hunger contractions of the empty stomach cause an increase reflex excitability of the cord (13). Similar effects are obtained on stimulation of the nerve endings in the gastric mucosa. Hot or cold water introduced directly into the stomach by the tube so as not to stimulate the mouth and esophagus cause a temporary augmentation of the knee jerk (fig. 2). The increase caused by the hot water (50°C.) is a trifle greater than that caused by the cold water. Practically the same results were obtained on both of us. No attempt was made to study the influence on the knee jerk of stimulation of the gastric mucosa during sleep.

Fig. 2. Record of knee jerk of A. J. C. *a*, 50 cc. water at 50°C. introduced into stomach through double tube; *b*, 50 cc. water at 5°C. into stomach. Showing augmentation of reflex excitability of spinal cord from temperature stimulation of the gastric mucosa.

SUMMARY

1. The normal gastric mucosa is devoid of pain and tactile sensibility, but protopathic temperature sensibility is present.

2. Chemical (and possibly mechanical) stimulation of nerve endings in the normal gastric mucosa gives rise to a sensation identical with appetite. The stomach mucosa may be said to be provided with a protopathic appetite sense.

3. The stimulation of the gastric mucosa does not contribute to the sensations of hunger and of fulness.

4. Stimulation of the gastric mucosa increases the reflex excitability of the spinal cord, and induces changes in vaso-motor tonus. The latter appears to be dependent on conscious cerebral processes induced by the gastric stimulation.

(1) HERTZ: The Sensibility of the Alimentary Canal, London, 1911.
(2) CARLSON: This journal, 1913, xxxi, p. 175.
(3) WEBER: Wagner's Handworterte d. physiol., viii, p. 497.
(4) BECHER: Zeitschr. f. Psychol., 1908, xlxi, p. 341.
(5) ZIMMERMAN: Mitteil a. d. Gernzg. Med. u. Chir., 1909, xx, p. 445.
(6) MACKENZIE: Symptoms and their Interpretations, 1909, p. 125.
(7) NYSTROM: Mitteil. a. d. Grenzg., Med. u. Chir., 1909, xxi, p. 125.
(8) ZUINCKE: Arch. f. exper. Pathol. and Pharm., 1889, xxv, p. 375.
(9) NEUMANN: Wien kl. Woch, 1905, xix, p. 923.
(10) ROUX: Maladies du Tube digestive, Paris, 1907, p. 375.
(11) HEAD, RIVERS AND SHERREN: Brain, 1905, xxviii, p. 99.
(12) CARLSON: This journal, 1914, xxxiii, p. xv.
(13) CARLSON: This journal, 1913, xxxii, p. 245.
(14) CARLSON: This journal, 1913, xxxi, p. 318.
(15) PAWLOW: The work of the Digestive Gland, London, 1902.

VARIATIONS IN IRRITABILITY OF THE REFLEX ARC

II. Variations Under Strychnine

E. L. PORTER

From the Laboratory of Physiology in the Harvard Medical School

Received for publication November 16, 1914

In previous papers[1] I have described the procedure by which the Martin method of measuring Faradic stimuli has been applied to a study of the variations in reflex irritability in the spinal cat. The fluctuations in threshold reported as occurring in asphyxia were large, the rise in threshold on withdrawing air amounting usually to more than 600 units from an initial threshold of about 5 units. The method permits equally well and with great accuracy the detection of changes of much less magnitude, down to a few hundredths of a unit in a good spinal preparation. On this account it was thought feasible to attempt the study of influences which would be expected to lower the threshold instead of raising it, although the changes on a reflex of low threshold would necessarily be small. The flexion reflex was used as in previous studies and was elicited by stimulation of the central end of the cut tibial nerve. In most experiments I have also followed the alterations in threshold of the crossed extension reflex and of reflex extension of the fore-limb. These two reflexes were elicited by single break shocks to the same nerve which gave flexion, namely, the tibial. As a control the irritability of a nerve muscle preparation on the fore-limb was determined at frequent intervals during some of the earlier experiments. The movement here was extension at the wrist, caused by stimulation of the peripheral end of a branch of the radial nerve. The experimental procedures adopted were never found to affect the threshold of this nerve-muscle preparation and in the later experiments

[1] E. L. Porter: This Journal, 1912, xxxi, p. 141, and 1913, xxxi, p. 223.

of the series its threshold was no longer followed. In all measurements on the flexion reflex the smallest detectable record of movement on the drum was taken to indicate that the threshold had been reached. When crossed extension and extension of the fore-limb appeared the movement, if any, was always comparatively large, hence to determine when the threshold had been reached they were observed directly without record on the drum. A 0.1 per cent solution of strychnine sulphate was the "excitant" used and the injections (generally 0.1 or 0.2 mgm. strychnine) were made into the jugular vein at intervals of from 2 to 10 minutes until the animal exhibited violent convulsions. It was never found possible to obtain reflex extension of the fore-limb before strychnine was given, although stimuli as high as 780 Z units have been applied in some cases. Crossed-extension was readily elicitable but its threshold in the undrugged animal, although fairly constant in any given case, varied widely in different preparations, running from a threshold approximately twice that for flexion to one a hundred times as high.[2]

The literature describes the action of strychnine as that of an excitant to the central nervous system. "The alkaloids of the strychnine group have a powerful stimulant action on the central nervous system, especially on the spinal cord."[3] "Die typische Wirkung aller dieser Alkalöide (strychnine group) besteht in der hochgradigen Steigerung der Reflexerregbarkeit . . . . und findet ihren Ausdruch in dem Tetanus." "Die Krampfanfälle werden . . . . durch die kleinsten, oft gar nicht mehr nachweisbaren Reize hervorgerufen, so dass sie scheinbar ohne alle Veranlassung eintreten."[4] "Die characteristische Wirkung des Strychnins sind die Steigerung der Reflexerregbarkeit und die Krampfanfälle, welche schliesslich zum Tode führen."[5] Sollmann[6] speaks of "increased irritability" and "increased excitability" in describing the action of the drug.

[2] Cf. Exp. of Nov. 15, Table I, p. 174 and Exp. of Dec. 6, Table II, p. 177.

[3] Cushny: Pharmacology and therapeutics, Philadelphia, 1901, p. 190.

[4] Schmiedeberg: Grundriss der Pharmakologie, Leipzig, 1906, pp. 108, 109.

[5] Magnus: Article "Strychnin" in the Real-Encyclopädie der gesamten Heilkunde, 4th edition, Berlin, 1907 and following.

[6] Sollmann: A Text-book of pharmacology, Philadelphia, 1908, pp. 143 and 876.

Storm van Leeuwen[7] using the flexion reflex in the spinal cat gave repeated stimuli of constant strength and noted the increased height of contraction following strychnine. He referred to this as "Steigerung der Reflexerregbarkeit." Just what is meant by heightened reflex irritability is not stated so definitely by other authors. They obviously intend to include the widespread and convulsive responses following strychnine and in addition they give the impression that impulses which in the unpoisoned animal are denied entrance to the cord are enabled after exhibition of strychnine to penetrate and produce reflex effects.

In 9 out of 15 experiments of my series in which the results were clear-cut the expected lowering in reflex threshold did occur. Following is a protocol of such an experiment.

Experiment of November 15, 1913

ELAPSED TIME	PROCEDURE AND REMARKS	THRESHOLDS IN Z UNITS		
		Flexion	Crossed extension	Extension of fore-limb
0.35		2.4		Inelicitable
0.45			5.2	Inelicitable
1.08		2.5	5.8	Inelicitable
1.22	Liquid removed from electrodes			
1.23		1.8	5.8	Inelicitable
1.37	0.1 mg. strychnine			
1.40		1.3	3.4	Inelicitable
1.44		1.2	2.1	Inelicitable
1.47	0.1 mg. strychnine			
1.49	Violent convulsions			
1.51		1.6	2.3	2.6
2.03		1.6	2.5	
2.05	0.1 mg. strychnine			
2.10		1.5	3.4	
2.12			1.6	1.6
2.14		1.4	1.8	
2.16				2.2

[7] W. Storm van Leeuwen: Archiv für die gesammte Physiologie, 1913, cliv, p. 307.

After one injection of strychnine a minimum of 1.2 Z units was reached from an initial threshold of 2.4 Z units, a drop of 50 per cent. The crossed extension reflex, starting with a threshold of 5.2 Z units reach 1.6 Z units, nearly the same minimum as for flexion. Reflex movement of the fore-limb also reached 1.6 Z units. The entire series of 9 experiments in which a drop in threshold occurred is summarized below.

TABLE 1

Summary of experiments in which a drop in threshold of the flexion reflex occurred following intravenous injections of strychnine. Thresholds are in Z units

DATE	REFLEX	THRESHOLD AT START	LOWEST THRESHOLD REACHED AFTER STRYCHNINE	THRESHOLD AT END OF EXPERIMENT	PERCENTAGE LOWERING OF THRESHOLD
March 17, 1913	Flexion	3.2	2.3	2.9	28
March 18, 1913	Flexion	5.3	4.4	6.1	17
June 6, 1913	Flexion	5.1	4.1	6.6	20
June 28, 1913	Flexion	3.3	2.3	2.4	30
July 8, 1913	Flexion	3.6	1.6	2.2	56
	Crossed-extension	28.8	1.8	2.7	94
October 25, 1913	Flexion	3.5	1.9	3.7	46
November 15, 1913	Flexion	2.4	1.2	1.4	50
	Crossed-extension	5.2	1.6	1.8	69
	Fore-limb extension		1.6	2.2	
November 28, 1913	Flexion	2.5	2.3	4.0	8
	Crossed-extension	4.5	3.3	8.4	26
	Fore-limb extension		5.4	7.6	
January 10, 1914	Flexion	4.1	2.8	3.4	31
	Crossed-extension	196.0	3.0	3.9	98
	Fore-limb extension		3.0	6.2	

But the lowering of the flexion reflex threshold after strychnine quite distinctly failed to occur in the remaining 6 of the 15 experiments. In the following protocol (abridged from the original) is an example of this result.

Following the injections of strychnine the threshold never went below 2.3 Z units, 0.7 of a unit above the initial value, despite the continuance of injections until convulsions occurred.

But although the flexion threshold was not lowered the drug was strongly operative on the thresholds of other reflexes. Crossed-extension was lowered from an initial threshold of 36

Experiment of November 30, 1913

ELAPSED TIME	PROCEDURE AND REMARKS	THRESHOLDS IN Z UNITS		
		Flexion	Crossed extension	Fore-limb extension
0.35		1.6	36.0	Inelicitable
1.04		3.2		Inelicitable
	Electrodes dried			
1.14		1.7	5.8	Inelicitable
1.20		2.1	7.2	Inelicitable
1.25	0.1 mg. strychnine			
1.30		2.3	5.7	Inelicitable
1.34		2.6		Inelicitable
1.35	0.1 mg. strychnine			
1.36	Vigorous twitchings			
1.38		2.6	4.0	Inelicitable
1.45		3.3		Inelicitable
	Electrodes dried			
1.47		2.1	5.0	Inelicitable
1.49	0.1 mg. strychnine			
1.50 to 1.53	Violent convulsions at intervals			
1.55		3.0	8.4	Inelicitable
1.59	Convulsions			18.5
2.01	Electrodes dried			
2.03		2.3	7.3	9.8
2.21	0.1 mg. strychnine			
2.23		2.2	2.8	4.3
2.26	0.2 mg. strychnine			
2.26	Violent convulsions			
2.27		2.2	3.0	
2.32	0.2 mg. strychnine			
2.33		2.2	2.3	2.6
2.37	0.2 mg. strychnine			
2.40		2.3	2.4	2.6
2.45		2.2	2.5	2.4

Z units to a minimum of 2.3 Z units. Extension of fore-limb, inelicitable at the beginning of the experiment, was finally lowered to a threshold of 2.4 Z units, after repeated injections of

strychnine and after the animal had been exhibiting convulsions for nearly an hour. The thresholds of the three reflexes were practically identical at the close of the experiment, flexion 2.2, crossed-extension 2.5 and extension of fore-limb 2.4. Here obviously the "heightened irritability" was not due to the greater ease with which stimuli entered the cord but to the greater ease with which such impulses elicited reflexes not normally thrown into activity by the particular group of sensory neurons stimulated. In some cases the thresholds of the three reflexes became actually identical for several readings, as the following protocol shows. Note the close correspondence of readings taken at 1.10, 1.17 and 1.20.

Experiment of January 17, 1914

ELAPSED TIME	PROCEDURE AND REMARKS	THRESHOLDS IN Z UNITS		
		Flexion	Crossed extension	Fore-limb extension
0.45		1.8	5.9	Inelicitable
0.47		1.9		Inelicitable
0.48	0.4 mg. strychnine			
0.48	Violent convulsions			
0.52		1.9	2.6	Inelicitable
0.56				3.0
0.59		2.3	2.6	2.6
1.02		2.2		
1.04			2.5	2.5
1.05	0.4 mg. strychnine			
1.10		2.4	2.4	2.4
1.17		2.6	2.6	2.6
1.20		6.5	6.5	6.5

The results seem to prove that synaptic resistances throughout the cord have been equalized. That this proof is not rigid will be indicated later (p. 182).

A summary of the 6 experiments in which no drop in reflex threshold was found follows (Table II). The lowest flexion threshold reached after strychnine is seen to vary from 0.1 to 2 Z units above the threshold in the undrugged animal.

A difficulty frequently encountered in determining thresholds was the short circuiting of a part of the current by blood or

lymph collecting between the electrodes. This necessitated a stronger current than was the case after the liquid had been removed. In the experiment of November 15 (p. 173) this was the case. At 1.22 liquid was removed from the electrodes and the threshold dropped from 2.5 Z units to 1.8 Z units. Similarly in the protocol of November 30 (p. 175), the threshold was lowered at 1.14, 1.47, and 2.03. Conceivably, then, a lowered threshold

TABLE II

Summary of experiments in which no drop in threshold of the flexion reflex occurred following intravenous injections of strychnine. Thresholds are measured in Z units

DATE	REFLEX	THRESHOLD AT START OF EXPERIMENT	LOWEST THRESHOLD REACHED AFTER STRYCHNINE	THRESHOLD AT END OF EXPERIMENT	PERCENTAGE RISE IN THRESHOLD
June 7, 1913	Flexion	3.6	5.6	7.2	52
June 30, 1913	Flexion	1.4	1.5	1.5	7
November 30, 1913	Flexion	1.6	2.1	2.2	31
	Crossed extension	36.0	2.3	2.5	
	Fore-limb extension		2.4	2.4	
December 6, 1913	Flexion	2.8	3.2	3.7	14
	Crossed-extension	300.0	5.2	94.8	
	Fore-limb extension		5.2	94.8	
January 7, 1914	Flexion	2.9	3.0	5.3	3
	Crossed-extension	5.6	3.0	5.9	
	Fore-limb extension		4.5	7.0	
January 17, 1914	Flexion	1.8	1.9	6.5	6
	Crossed-extension	5.9	2.4	6.5	
	Fore-limb extension		2.4	6.5	

following strychnine might commonly be masked by the presence of liquid between the electrodes. This has been shown to be improbable in an experiment in which liquid was prevented from accumulating on the nerve by a small pledget of cotton between the electrodes and in contact with the nerve. Immediate injections of strychnine were followed promptly by convulsions, but the threshold after strychnine stood 1.0 Z unit above its initial value. It was conceivable that the cotton had dried the

nerve and rendered it less irritable. The cotton was therefore wet with saline and left for five minutes. It was then removed, the surface of the nerve dried, and the threshold again determined. It was still 0.2 Z unit higher than before strychnine. The experiment was performed rapidly, only 22 minutes elapsing between the first and the final determinations. The conclusion seems justified that thresholds of the flexion reflex in the spinal cat frequently occur which cannot be lowered by strychnine.

That this conclusion holds also for the spinal frog is indicated by results obtained by Elrington.[8] He secured reflex contractions of the gastrocnemius muscle by stimulation of the ninth dorsal nerve root and measured the threshold before and after strychnine. His measurements are given in the units of the Kronecker scale and the strength of the primary current is not stated so that his results and my own are not comparable directly; percentage changes, however, can be compared. In eight out of twenty cases he found either no change in threshold after strychnine or a rise varying from 55 to 900 per cent of the initial value. I found in the cat in six out of fifteen cases a rise of from 3 to 52 per cent (Table II). In twelve cases he found a drop of from 17 to 95 per cent. I found similarly in nine cases a drop of from 8 to 56 per cent. Percentage alterations were thus much greater with his animals than with mine, but the proportion of cases in which strychnine failed to cause lowering was the same as I found, namely one out of three. Provided he used a primary current of the same strength in all cases his animals also show wider variations in normal threshold, that is, before strychnine. His values for initial thresholds vary from 60 to 2000 units on the Kronecker scale; mine from 1.4 to 5.3 Z units, Martin calibration (Tables I and II). Such wide variations are like those I found for the crossed-extension reflex, namely, 4.5 to 300 Z units (Tables I and II). This suggests that in using the reflex contractions of the gastrocnemius, Elrington may have been dealing with a reflex less simple than flexion or else the simplest reflexes in the frog have less uniform thresholds than corresponding ones in

[8] Elrington: Zeitschrift für allgemeine physiologie, 1914, xvi, p. 115.

the cat. It would appear also from Elrington's results that reflex contractions of the gastrocnemius vary in threshold from moment to moment for he frequently gives the value as "0–30," "100–200," and so forth. I have always found the threshold for flexion in the spinal cat very sharp and easy of determination, with an error of perhaps 1 or 2 per cent.

If the flexion reflex threshold be raised by ether, strychnine promptly lowers it. Figure 1 is a graphic record of such an experiment. Ether was administered by shunting a part of the air used for artificial respiration through an ether bottle. The

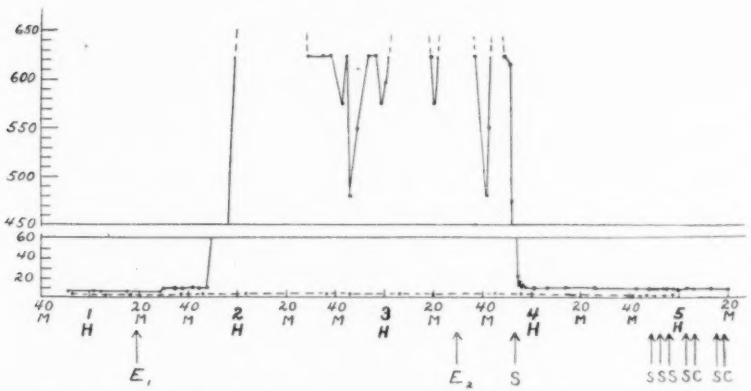


Fig. 1. Effect of injections of strychnine on a reflex threshold which had been raised by ether. E_1, administration of ether in varying amounts begun; E_2, amount of ether administered was left unchanged from this point to the end of the experiment; S, injections of strychnine of 0.2 mgm. or 0.3 mgm.; C, convulsions. Ordinates, values of Z; abscissae, elapsed time in hours and minutes. Continuous line, reflex; broken line, nerve-muscle preparation. A portion of the vertical part of the record from ordinate 60 to ordinate 450 has been omitted (Exp. of July 22, 1910).

proportion of ether to air could be varied at will. The original purpose was to raise the threshold only moderately, to an "ether plateau" so to speak, and hold it at that point for a considerable interval during which the strychnine could be injected. Regulating the administration of the ether so as to being this about was found impossible; the threshold either remained low or rose

to a great height and even suddenly and completely disappeared, to reappear as suddenly when the amount of ether was slightly diminished. Accordingly the attempt to obtain the ether plateau was given up, and at 3.30 the amount of ether was left at a certain definite ratio to the air and this proportion was unchanged to the end of the experiment. At 3.52 with the threshold of the reflex at 612 units, 0.3 mgm. of strychnine was injected. One minute later the threshold was 14 units and in four minutes down to 8 units. This was only 3 units above the threshold at the beginning of the experiment three hours before, al-

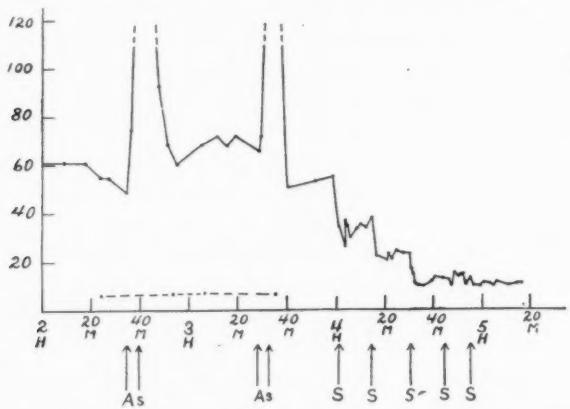


Fig. 2. Lowering by strychnine of a reflex threshold high from the effects of asphyxia. The lowering takes place in successive stages. *As*, asphyxia (artificial respiration discontinued), *S*, injections of strychnine (0.2 mgm. doses). Legend otherwise as in figure 2 (Exp. of Nov. 2, 1911).

though in the meantime it had been for two hours not lower than 480 units. Further doses of strychinne failed to affect the threshold more than slightly although convulsions were produced. In another experiment of the same kind there was a drop in consequence of injections of strychnine but this was followed by a rise as the ether made itself effective against the strychnine. A second dose of strychnine again resulted in a drop and this was repeated a third time.

The threshold can be lowered when it is high in consequence of influences other than drugs. Figure 2 is the plot of an experi-

ment in which the reflex threshold was high following asphyxia. Five successive doses of strychnine were given. Each of the first three doses forced the threshold to a point lower than it had held preceding the dose. After the fourth and fifth doses, however, although the threshold was lowered, it never went below the minimum point reached after the third injection. A similar lowering by stages has been observed when strychnine has followed ether.

Assuming that strychnine is the strongest "excitant" which could have been used, this series of experiments indicates that the flexion reflex is frequently in the highest state of irritability of which the mechanism is capable. This is not surprising. Flexion of the leg is a protective reflex in frequent use. To be

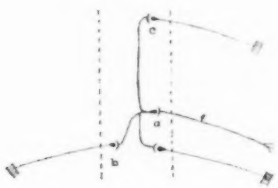


Fig. 3. Exrlanation in text.

most serviceable to the organism it must be set on a "hair-trigger." Here if anywhere amongst spinal reflexes we might expect to find a threshold at its lowest possible level.

Figure 3 is a modification of Sherrington's[9] diagram of neurones and synapses concerned in the production of typical spinal reflexes. Suppose (a) to represent the synapse of highest threshold in the flexion reflex arc, (b) the corresponding synapse for crossed extension, and (c) that for extension of the fore-limb. If we assign to (a) a resistance of 1, then according to my determinations (b) will have a value of 1.8 to 107, dropping to 1 or nearly so after strychnine. Synapse (c) will have a resistance infinitely high in the undrugged animal but after strychnine its resistance, like that of (b), will become approximately 1.

[9] Sherrington: The integrative action of the nervous system, New York, 1906, p. 156.

There is nothing in this series of experiments to prove that (b) and (c) may not acquire even less resistance than (a). When, in the experiment of January 17 (p. 176), at 1.10, flexion, crossed-extension and extension of fore-limb all respond to a stimulus of 2.4 Z units and not to a less stimulus it may mean merely that a flexion synapse having a resistance of 2.4, as (a), must be crossed before crossed-extension and fore-limb movement appear. But synapses (b) and (c) may conceivably have a still lower threshold.

The effects on the nervous system of strychnine and of asphyxia in its early stages are closely similar. "Increased reflex irritability" has frequently been reported in consequence of lack of oxygen. I[10] found no clear evidence of a lowered reflex threshold in asphxia although the usual convulsions, superficially resembling those of strychnine, were always observed. Perhaps, then, in asphyxia the increased reflex irritability is due not so much to the lowering of reflex thresholds toward entering stimuli as to the lowering of thresholds of many or all reflexes toward stimuli which have already entered the cord.

SUMMARY

In the spinal cat thresholds of the flexion reflex are frequently present which are not lowered by strychnine even when administered in doses causing convulsions.

Thresholds of the flexion reflex more commonly, however, are lowered by strychnine, the drop varying from 8 to 56 per cent of the original height of threshold.

The threshold of the crossed extension reflex, normally from 2 to 100 times as high as the flexion threshold, is lowered nearly or quite to the flexion threshold by strychnine. The threshold for reflex extension of the fore-limb, a movement inelicitable in the undrugged animal, is similarly lowered.

A flexion threshold which is abnormally high following administration of ether, or in consequence of asphyxia, is lowered by strychnine to approximately the normal level. The lowering may take place in stages following successive doses of the drug.

[10] E. L. Porter: This Journal, 1913, xxxi, p. 241.

CONTRIBUTIONS TO THE PHYSIOLOGY OF THE STOMACH

XX. The Contractions of the Rabbit's Stomach During Hunger

FRED T. ROGERS

From the Hull Physiological Laboratory of the University of Chicago

Received for publication November 16, 1914

The work of Carlson[1] on man and dogs has confirmed the conclusion of Cannon and Washburn[2] that the sensation of hunger is intimately associated with contractions of the empty stomach. Contrary to the common statement that the empty stomach is quiet and at rest, it can be easily shown that the stomach is most active when empty. But since the stomach of herbivorous animals, and especially that of the ruminants, is under normal conditions never empty and these animals undoubtedly feel hunger, the question arises of how hunger is to be explained in them. At the suggestion of Professor Carlson the subject was studied in the rabbit.

Gastric fistulas were made in the rabbits by opening the abdominal cavity about one inch to the left of the mid-ventral line and as close to the costal border as possible and suturing the muscularis of the fundic portion of the stomach to the peritoneum and oblique muscles and then, the gastric mucosa to the skin. These fistulas were made rather small so that there would be little leakage of gastric juice or loss of food. At times a small rubber tube was inserted into the stomach and left there for longer or shorter times to prevent closing of the fistula. Within thirty-six hours after the operation animals are usually feeding. Such animals were kept in the laboratory for periods of two to six

[1] Carlson: This Journal, xxxi, p. 175.

[2] Cannon and Washburn: This Journal, xxix, p. 441.

weeks. Rabbits with these fistulas, if properly taken care of, are in as good condition as normal animals. In only two or three cases were gastro-intestinal troubles noted and these were in animals which had been submitted to prolonged starvation and had nasal and pulmonary infections. About eighteen rabbits were used in these tests.

Graphic records were made using the rubber balloon method described by Carlson. The manometers and writing levers were adjusted very carefully so as to show all changes of the stomach movements. Care was taken to use as low a pressure in the writing apparatus as would clearly record the movements. This was 2 to 4 cms. of chloroform. The relative sizes of the distended balloon and stomach are shown in figure

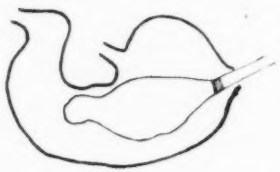


Fig. 1. The distended balloon in position in the rabbit's stomach. From a life-size tracing.

1. In this particular instance, the balloon had been inserted thirty-six hours before death and the pressure within it had not been changed, from the exterior, within that time. The space between the balloon and the stomach wall was filled with a dirty green residue.

Medium size adult rabbits were used in this set of experiments. In no case did any animal survive a period of continuous starvation of more than seven days But since they were frequently starved for several days and then fed for varying lengths of time and again starved, this short period of life on continuous starvation is not surprising. However a review of the literature shows that only large and heavy animals can stand more than two weeks deprivation of food.[3] Plenty of water was always given

[3] Luciani: Physiologie des Menschens., 1911, iv, p. 445.

the animals. But the surprising result appeared that in animals that had died of starvation, still there were always considerable amounts of residue in the stomach. In the moist condition in which this was removed from the stomach it weighed from 8 to 13 grams. Normally the moist contents of the adult rabbit's stomach weighs 90 grams or more. It therefore seemed that the rabbit was unable to completely empty its stomach.[4] Then it was noticed, that soon after being deprived of its usual food, the animal provides a substitute by eating its own excreta. This is merely confirmation of what others have noticed. Putting the animal in a wire bottom cage does not prevent this. The only practicable way found to prevent it was to enclose the animal in a cage so fitted with a lid, that the rabbit's head was held outside, its body inside the cage. No doubt such a position proved very irksome to the rabbit, but after becoming accustomed to the position the activities of the stomach were not inhibited. In an animal so fixed, the stomach, as noted by Swirski,[5] empties itself in about twenty-four hours. Nevertheless, during normal conditions of life, most certainly the rabbit's stomach is never empty.[6]

Since normally the stomach is never empty, it is to be expected that gastric digestion peristalsis will always persist. Apparently this is true up to a short time before death. But as the period of starvation is prolonged, the stomach contractions are altered. Instead of being the gentle peristalsis of normal digestion, they become relatively powerful contraction waves which rapidly follow one after the other with a tendency for each peristaltic wave to pass into a short period of tetany (fig. 2). There is no indication of rest or periodicity to these stronger contractions until a short time before death from starvation. Auer[7] has pointed out that during normal peristalsis in the rabbit, the stomach is incessantly active. During hunger the strength of these contractions is accentuated.

[4] Heidenhain: Arch. f. des. ges. Phys., xliii, Sup. heft., 1888, p. 38.
[5] Swirski: G. Arch. f. exp. Path., 1898, 41, p. 143.
[6] Heidenhain: Loc. cit.
[7] Auer: This Journal, xxiii, p. 165.

Following this period of increased activity there comes a period of depression. This is coincident with marked weakness on the part of the rabbit or even with the approach of coma. The decline in the vigor of the stomach activities comes on gradually. The contractions become weaker, of shorter duration, and alternating with short periods of rest (fig. 2). In the last stages of starvation there may occur prolonged contractions or periods of tetany lasting from two to three minutes. This confirms the observations of Patterson[8] on dogs, that the hunger contractions persist nearly up to the time of death.

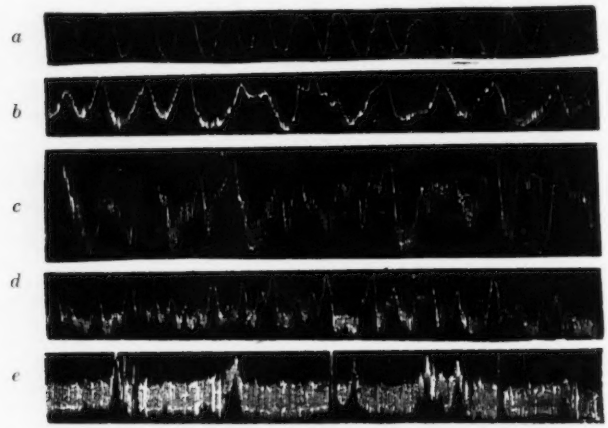


Fig. 2. Contractions of the rabbit's stomach. *a*, normal digestive peristalsis; *b*, after 24 hours starvation; *c*, after 75 hours starvation; *d*, after 92 hours starvation; *e*, after 110 hours starvation. The animal died a few hours after this tracing (*e*) was taken.

There are also differences between the normal and the hungry rabbit in the ease with which the stomach contractions are inhibited (fig. 3). This may not occur until the third or fourth day of starvation, unless precautions are taken to exclude the animal from its excreta. Water, 10 per cent alcohol, sugar solutions, 0.2 per cent–0.4 per cent hydrochloric acid, and fruit juices inhibit or weaken the contractions in the hungry animal. This was deter-

[8] Patterson: This Journal, xxxiii, p. 423.

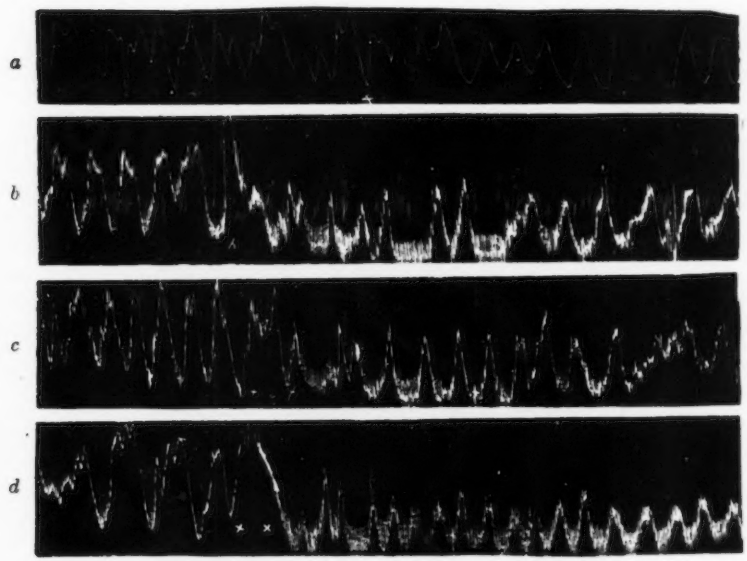


Fig. 3. *a*, 10 cc. of 0.25 per cent hydrochloric acid put into the stomach during normal digestion peristalsis; *b*, 10 cc. of 0.25 per cent hydrochloric acid put into the stomach of a hungry rabbit; *c*, 10 cc. of water put into the stomach of a hungry rabbit; *d*, 10 cc. of 10 per cent alcohol put into the stomach of a hungry rabbit. Note the inhibitory effect of these solutions on the hunger movements.

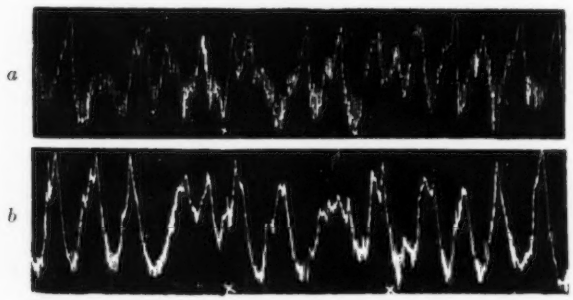


Fig. 4. Hunger contractions of a rabbit on fourth day of starvation. *a*, at X-X chewing carrot moistened with 0.4 per cent hydrochloric acid; *b*, at X-X chewing sweet carrot. No inhibition.

mined either by swallowing (water) or by introducing these solutions into the stomach through a small rubber tube passing through the fistula. In these tests especial care was taken not to excite the rabbit.

The sight, smell, taste, or chewing without swallowing, of such foods as cherries, carrots, apples and carrot leaves moistened with sugar, acid, or quinine do not inhibit the stomach

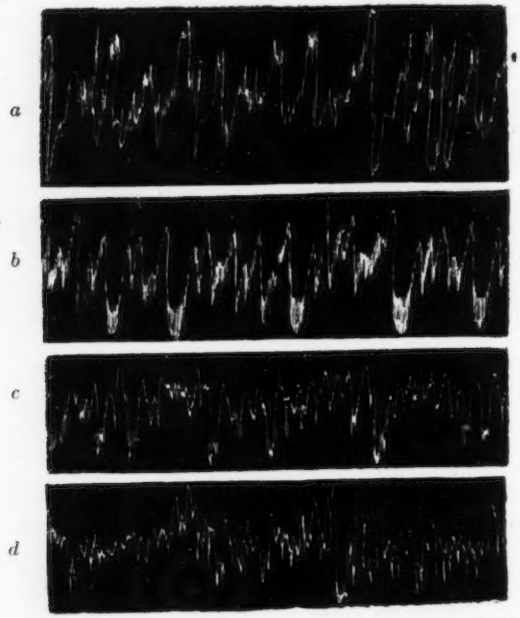


Fig. 5. Hunger contractions of a rabbit which was excluded from its feces. *a*, *b*, *c*, *d*, respectively contractions after 12, 24, 48 and 52 hours of starvation.

contractions (fig. 4). The relative difficulty of inhibiting the stomach contractions even when the animal is undoubtedly hungry, is in striking contrast to the ease with which this occurs in man or in dogs.[9]

Excitement not only inhibits these hunger contractions, if such they may be called, but causes a marked lowering of the

[9] Carlson: This Journal, xxxii, p. 389.

gastric tonus. In fact tonus variations were frequently seen during hunger but not commonly during normal digestion peristalsis. May not the increasing muscle tone of the stomach, as starvation is prolonged, play a part in causing the sensation of hunger? Certainly a state of greater contraction of the stomach on the substances remaining within it, as starvation is prolonged, is the first apparent change (fig. 6). Rabbits will show signs of hunger, such as restlessness, gnawing dry wood and eating cotton, before the character of the movements as recorded by this method shows any striking change from the normal digestion peristalsis. The increased activity of the stomach that later appears is no doubt also accompanied by psychic

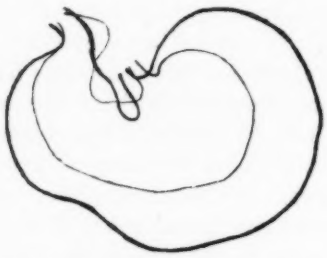


Fig. 6. Superimposed lifesize tracings of the stomachs of two adult rabbits; the larger represents the stomach of a rabbit killed after abundant eating; the smaller the stomach of a rabbit which died of starvation, but had been permitted to eat its own feces.

changes. The appearance of gastric tetany if the animal is still able to move about, is marked by restlessness.

In order to determine whether or not the stomach of the rabbit when it contains no food is quiescent, the animal was so caged as to be excluded from its feces. The only difference observed between an animal so caged and one free to move about, was that the increased vigor of the stomach contractions comes on sooner in the former. In about twelve hours after feeding the contractions become very much stronger than normal digestion peristalsis and this activity both of contractions and tonus variations persists after the stomach has emptied itself of food (fig. 5). What such an animal must feel can only be imagined

by one who has felt the periods of hunger tetany in his own stomach. The inhibitory reflexes are similar to those of the normally hungry rabbit.

SUMMARY

During starvation of the rabbit the stomach is never empty unless precautions are taken to prevent the animal from eating its feces. In the latter case the stomach of the adult rabbit empties itself in about twenty-four hours.

During starvation of the rabbit, the stomach activities are augmented and the reflexes acting on the gastric motor mechanism are different from those of normal digestion peristalsis. But there are no sharp dividing lines between the normal peristalsic contractions occurring during digestion and those occurring during hunger. *It would thus seem that in the rabbit at least, the gastric hunger contractions are intensified digestive peristalses.*

The *empty* stomach of the rabbit shows very strong and rapidly repeated contractions and marked tonus variations.

In the hungry rabbit there is a state of increased tonus in the stomach musculature. Tonus changes are more marked and occur more frequently during hunger than during normal digestion peristalsis.

The contractions of the stomach occurring during hunger in the rabbit are not inhibited by the sight, smell, taste or chewing of food.

The contractions occurring during hunger are inhibited or weakened by the introduction into the stomach of small quantities of water, weak acids, alcohol or sugar solutions. Normal peristalsis is not inhibited by the same amounts of these solutions.

Excitement (central nervous influences) inhibits the stomach contractions and causes a lowering of the tonus of the stomach musculature.

The relation of the hunger contractions to normal peristalsis is being studied further in man and dogs as well as in rabbits.

My hearty thanks are due Professor Carlson for his help and suggestions; to the laboratories of Baylor University for material; and to Drs. Aynesworth and Dudgeon for aid in the operations.

CONTRIBUTIONS TO THE PHYSIOLOGY OF THE STOMACH

XIX. Reflexes from the Intestinal Mucosa to the Stomach

E. H. BRUNEMEIER and A. J. CARLSON

From the Hull Physiological Laboratory of the University of Chicago

Received for publication November 16, 1914

The tonus and the contractions of the empty stomach (man and dog) are temporarily inhibited by stimulation of nerves in the mouth, in the oesophagus, and in the gastric mucosa itself.[1] Can the tonus and hunger contractions of the empty stomach be influenced reflexly by stimulation of the intestinal mucosa? The answer to this question might explain the diminution or abolition of hunger by the introduction of chyme into the intestine.[2] If such reflex relations exist, it is obvious that the intestinal mucosa must be an important factor in the control of the gastric tonus and hunger mechanism.

Boldireff[3] reports that acids in the intestine inhibit the periodic activity of the empty stomach. The inhibition was not obtained by water or alkaline solutions. In fact Boldireff appears to imply that the periodic contractions of the empty stomach may be initiated by the introduction of a solution of 0.3 per cent Na_2CO_3 into the intestine. He therefore concludes that the reflex inhibition is due to an acid stimulation of nerves in the intestinal mucosa.

EXPERIMENTAL METHOD

In this work we used 24 young female dogs. Intestinal fistulæ were made by Abbe's lateral anastomosis in the first loop of the small intestine below the pancreas, the cephalad end

[1] Carlson: This Journal, 1913, xxxi, p. 212; xxxii, p. 245 and p. 389.

[2] Busch: Arch f. Pathol. Anat. u. Physiol., 1858, xiv, p. 140.

[3] Boldireff: Zentralbl. f. Physiol., 1904, xviii, p. 989.

being sutured into the abdominal wall and left open to the exterior. The gastric fistulæ were made after recovery from the first operation.

In another group of dogs a Tiery fistula was made but no gastric fistula, the recording apparatus being introduced into the stomach through the oesophagus.

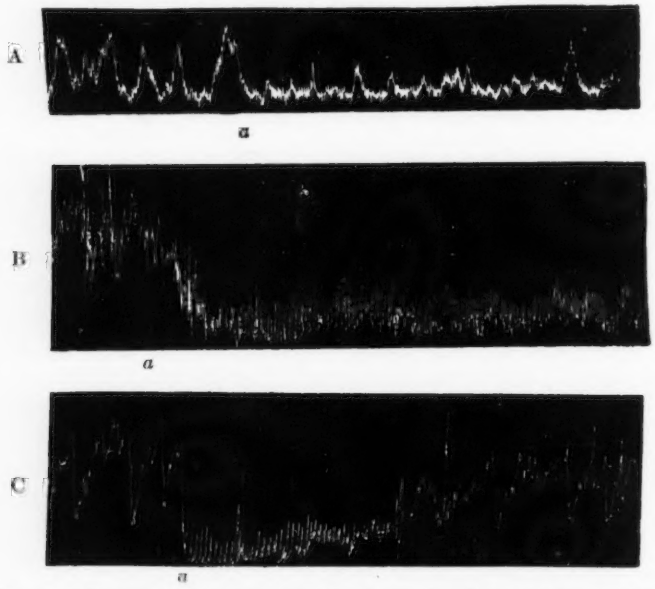


Fig. 1. Tracing showing tonus and hunger contractions of the empty stomach of dogs. A, 10 cc. of gastric juice introduced into small intestine at *a*. B, 10 cc., 0.5 HCl introduced into small intestine at *a*. C, 10 cc. 1 per cent Na_2CO_3 introduced into small intestine at *a*. Showing reflex inhibition of the tonus and the hunger contractions of the empty stomach by chemical stimulation of intestinal mucosa.

In a third group the gastric fistula was made near the pyloric end of the stomach. Through this fistula a small stomach tube was passed through the pylorus into the small intestine for varying distances. This tube was kept in the gut throughout the experiment for the introduction of the liquids into the in-

testine. The recording balloon was passed into the stomach either through the gastric fistula or through the oesophagus.

In the last group of dogs the vagi and splanchnic nerves were cut, and after recovery from the operation, gastric fistulæ were established in the antrum pylori. In all tests on this group the fluids were introduced into the intestine by means of a tube passed through the pylorus, and the stomach balloon was passed down through the oesophagus.

The following solutions were introduced into the intestine in 10 cc. quantities, in most cases at body temperature:

1. Normal gastric juice (dog, man).
2. 10 per cent Witte's peptone in 0.2 per cent HCl.
3. Pepsin in 0.2 per cent HCl.
4. Hydrochloric acid (0.1 per cent to 0.5 per cent).
5. Saturated H_2CO_3 solution.
6. Sodium carbonate (0.2 per cent to 1 per cent).
7. Glucose (neutral solution).
8. Neutral olive oil.
9. Fresh milk.
10. Water.
11. Mechanical stimulation of the intestinal mucosa (glass rod or rubber tube).

RESULTS

When the vagi and the splanchnic nerves are intact *all mechanical and chemical stimulations of the intestinal mucosa cause inhibition of the gastric tonus and hunger contractions.* The effect of a purely mechanical stimulation (rubbing the mucosa with a glass rod or rubber tube) is the most transitory. In general pure gastric juice and the 0.5 per cent HCl cause the longest inhibition. The acid peptone solution followed these closely. The weaker acids produced inhibition of less duration. Saturated carbonic acid solution did not give quite so distinct an inhibition as the other acids. Inhibition with pure gastric juice and the acid-peptone mixture varied in duration from three to twenty minutes, depending apparently largely on the condition of the animal at the time. The sodium carbonate solution caused

inhibition of less duration than acid mixtures but of longer duration than the water or the neutral mixtures in general. However, the longest inhibition obtained in any one experiment was produced by 10 cc. of milk in the gut. In this case the inhibition lasted thirty minutes. Ordinarily, neutral solutions produced a longer inhibition than the mechanical stimulation by moving the soft rubber tube or the stomach tube in the intestinal fistula.

In the animals with the vagi and splanchnic nerves severed the above substances still caused reflex inhibition of the empty

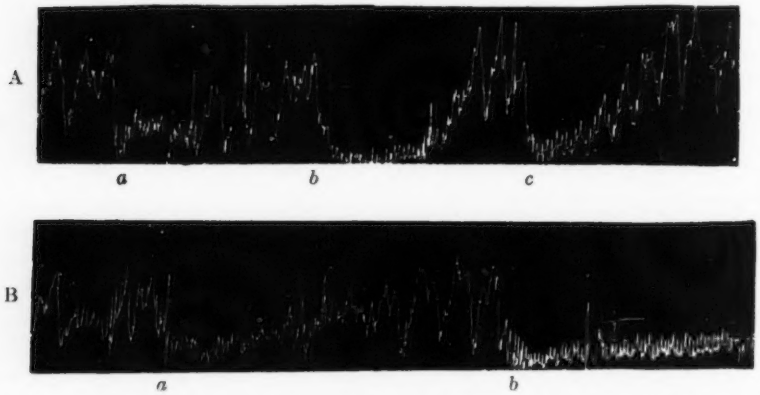


Fig. 2. Tracings from the empty stomach of dogs. A, *a*, mechanical stimulation of intestinal mucosa (gently moving a rubber tube in the lumen); *b*, 10 cc. water introduced into the intestine; *c*, 10 cc. of 0.3 per cent Na_2CO_3 introduced into the intestines. B, *a*, 10 cc. of 10 per cent peptone in 0.2 per cent HCl introduced into intestine; *b*, 10 cc. fresh milk introduced into the intestine. Showing temporary inhibition of tonus and hunger contractions of the empty stomach by mechanical and chemical stimulation of the intestinal mucosa.

stomach from the intestinal mucosa, but the latent period of the inhibition was greatly prolonged, the degree of the inhibition less, and the duration of it much shorter than in the normal animals.

It is therefore clear that this inhibition of the tonus and contractions of the empty stomach by chemical and mechanical stimulation of the intestinal mucosa involves both long or cen-

tral and short or local reflex paths, a situation similar to that found in the gastric mucosa itself.[4]

The precise rôle of this reflex in the control of the gastric hunger mechanism in the normal animal must be determined by further investigation. It is probably a factor in the diminution or absence of hunger in cases of enteritis, intestinal obstruction, and constipation.

CONCLUSIONS

(*a*) Gastric juice, chyme, acids, alkalies, water, milk and oil introduced into the small intestine inhibit gastric hunger contractions and gastric tonus for varying periods.

(*b*) This inhibition of these gastric hunger contractions and tonus is due partly to mechanical, partly to chemical stimulation of the intestinal mucosa. The chemical stimulation produces the greatest effect.

(*c*) This inhibition of gastric hunger contractions takes place apparently primarily by the "long" or central reflex path, but "short" or local reflex paths in Auerbach's plexus are also involved.

[4] Carlson: This Journal, 1913, xxxi, p. 212; xxxii, p. 245 and p. 389.

CONTRIBUTIONS FROM THE ZOÖLOGICAL LABORATORY OF THE MUSEUM OF COMPARATIVE ZOÖLOGY AT HARVARD COLLEGE. No. 258

A NOTE ON THE PHYSIOLOGY OF THE CUVIERIAN ORGANS OF HOLOTHURIA CAPTIVA LUDW.[1]

W. J. CROZIER

Received for publication November 18, 1914

CONTENTS

I. Introduction.
II. Stimuli which lead to Cuvierian organ expulsion.
III. Mechanism of discharge.
IV. The adhesive property.
V. Summary.

I. Concerning the function and mode of action of the Cuvierian organs of holothurians there is available very little experimental evidence.[2] The following observations upon the physiology of these structures in one of the Bermudan species of Holothuria may therefore be of interest.

Holothuria captiva does not bury itself in mud or sand, but is to be found (during the day-time at least) only on the under surface of slabs of stone, in places where numbers of such stones occur piled together just under low-water level.[3] It clings to the rock surfaces with such tenacity that frequently numbers of pedicels must be torn before the animal can be removed. During the process of collection there almost invariably occurs the expulsion of some of the Cuvierian organs, which in this species

[1] Contributions from the Bermuda Biological Station for Research. No. 37.

[2] For an account of the views of varius writers see Minchin (1892) and Mines (1912).

[3] Such localities were: Hungry Bay, near the entrance; Bluck's Point; and the old break-water near Walsingham, Castle Harbor. Previous papers (Crozier, 1914 a [?] and 1914 b) contain data on the behavior of these animals. The present account is based upon the experiments during the summers of 1913 and 1914, at the Bermuda Biological Station.

are very abundant (cf. Clark, 1901, p. 342). I have never seen a case in which the tubes were in an extruded condition when the rocks bearing H. captiva were turned over under water, though in all several hundred animals were observed in their natural position. I can therefore offer no suggestion as to their rôle in the normal economy of the animal.

II. It has generally been stated that holothurians possessing Cuvierian organs expel them when the animal is "irritated;" presumably mechanical stimulation of an intense character is meant. H. captiva was observed to discharge its tubes when the back or side was stimulated by repeated gentle touches with a blunt instrument, or when the papillae or tentacles were pinched. The brim surrounding the dorsally situated anus became at the same time elevated into a funnel-shaped tube, the opening of which was, in a general way, directed toward the irritated area. The number of organs discharged varied with the intensity and duration of stimulation. The individual threads were frequently as much as ten times the length of the animal producing them. Usually, but not always, one end of the Cuvierian organ remained attached to the cloaca. The general appearance and behavior of the threads followed very closely the account given by Minchin (1892) of the discharged Cuvierian organs of H. nigra.

The application of solutions of a variety of "irritating" substances to the surface of H. captiva did not lead to the discharge of the organs. Half-cubic centimeter volumes of inorganic acids ($\frac{M}{100}$), phenol ($\frac{M}{100}$), turpentine, essential oils, etc., were used. To such solutions the animal reacts vigorously, but not by the expulsion of Cuvierian organs.

A gradual increase in the temperature of the seawater, up to 40°C., did not induce the discharge of the tubes. I have previously noted, in H. surinamensis, the absence of a temperature sense (Crozier 1914a [?]).

Immersion in diluted seawater or in rain water did not lead to Cuvierian organ discharge.

I have elsewhere shown (Crozier, 1914 b) that light has a toxic action on these animals. Holothurians exposed to the

direct rays of the sun sometimes discharged a few Cuvierian organs, but after being in bright sunlight for a short time (about 15 minutes) they passed into a moribund condition, in which no tubes were expelled in response to mechanical stimulation. When kept in laboratory aquaria shielded from the light, the partial stagnation of the water was accompanied by the discharge of some of the tubes long before auto-evisceration made its appearance.

It would therefore seem that the normal stimulus for the discharge of these organs is of a tactile or mechanical nature, since it is only in response to such stimuli that the tubes are discharged promptly and elongated to any extent.

III. Mines (1912) found that the discharge of the Cuvierian organs of H. nigra was accompanied by an increase of internal pressure comparable to that which he found sufficient to produce the extension of the isolated tubes, and hence concludes that the tubes are extended by water which is forced into them, in accordance with the view of Hérouard (Delage et Hérouard, 1903). If Holothuria captiva be plunged into water at temperatures between 40°C. and 60°C., the animal contracts somewhat and several Cuvierian organs are usually expelled, though they are coagulated before they are extended beyond a centimeter or so. The internal body pressure may also be artifically raised by pressing on the holothurian's back, in which case the tubes also make their appearance. If the pressure is gentle, they are forced out and break off as small cigar-shaped bodies tapering at both ends, about 8 to 10 mm. long and 0.2 mm. in diameter (fig. 1). By pressing on the animal suddenly and with considerable force the tubes may be caused to be shot out in typical fashion. When placed in rain-water at 36°C., H. captiva dies in less than ten minutes; pressure caused the expulsion of Cuvierian organs from animals killed in this way, but they were never completely extended.

There are a number of facts which indicate that an increase in internal pressure is not the only, nor even the principal factor producing the discharge and elongation of the tubes. When

H. captiva undergoes auto-evisceration,[4] the rupture of the cloacal wall is accompanied by a considerable increase of internal pressure, but it is only rarely that any Cuvierian organs are even partially discharged during this process. Moreover, and this is a factor which has apparently been overlooked, there proceeds from the anus of an animal under experimental pressure, and also in the normal discharge of the organs, a fairly strong current derived from water previously held in the respiratory trees. The direction of this current was observed and its force estimated in water colored with carmine particles. It was clearly evident that the current served to direct and elongate the Cuvierian organs. By this means the organs are generally directed away from the animal discharging them, which is thus prevented from entangling itself. If the unextended organs secured by gentle pressure on a holothurian were taken up in a wide-mouthed pipette and ejected therefrom under the water, they became greatly elongated. A stream of water directed upon an unextended tube lying in a glass dish also produced this elongation, especially when one end of the tube had become attached by its sticky secretion; the latter condition corresponds almost exactly to the state of affairs during the normal discharge of the organs.

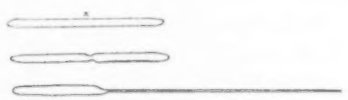


Fig. 1. Showing the reaction of a single Cuvierian organ—obtained by pressing on a Holothuria captiva—to tactile stimulation at the point x. The time occupied by the reaction was in this case 3 seconds. Magnified 2 diameters.

The cloacal stream has also another effect. As is noted by several writers (see Mines, 1912), the Cuvierian organs possess an intrinsic elongating mechanism, consisting of circular muscle

[4] Even when kept in well aerated seawater aquaria protected from the light, holothurians will eviscerate after about four days. This does not happen, however, until all ingested material has been removed from the gut by defecation. It is therefore possible that one form of the stimulus to evisceration originates in the gut, and it may be suggested that possibly the stimulus results from the autolysis of the intestine, due to the accumulation of digestive enzymes, which under normal conditions are continually removed, since the gut is always full.

fibers and some longitudinal ones. The structure of the tubes of H. captiva agrees very well with Ludwig's account of these organs in H. poli (Ludwig, 1892, p. 173). The short tubes above described as being ejected under gentle pressure may be kept alive for some time in seawater. If they are touched, as with the tip of a fine glass rod, they constrict at about the middle point and from this level proceed to elongate in both directions. Sometimes only the proximal half became constricted and elongated, the distal half—corresponding to the "head" of the organ as described by Minchin (1892) and Russo (1899)—retaining its original form. They also reacted locally by constrictions. Inasmuch as the tubes, as normally discharged, travel faster during part of their course than does the cloacal stream, the water current probably stimulates their extension by the self-contained mechanism. These holothurians when pressed upon sometimes emitted tubes as those above described, which were swirled about by the cloacal stream and stimulated to extension; whereas other tubes of the same sort upon which the cloacal stream did not impinge remained unextended. I therefore believe that the increased body pressure observed by Mines is primarily related to the production of the cloacal stream, and only secondarily implicated in the discharge and elongation of the tubes.

There are, then, at least three factors involved in the discharge and extension of the Cuvierian organs; the internal body pressure, the mechanical and stimulating action of the cloacal current, and the intrinsic extending mechanism of the tubes.

The nervous path of the stimuli leading to Cuvierian organ expulsion is not known. With the object of discovering whether the oral nerve ring took part in the matter, I experimented upon animals in which the anterior end had been cut off. The tubes were sometimes ejected by individuals so prepared, but never in a fully extended condition. The result is not decisive, since animals with the anterior end intact frequently presented the same condition after they had been in the laboratory for some time. With such animals, in which the general level of tone is very low, the unextended tubes could be obtained by pressure very much more easily than with the specimens in good

condition. This points to the conclusion that the normal release of the Cuvierian organs includes the action of the nervous system, though perhaps in an indirect way.

IV. When the tubes are in the discharged condition they are very sticky. In tubes ejected in water above 40°C. the stickiness is not so evident, even when the tubes are drawn out. Neither are they sticky when extruded into rain water. The adhesive property is somewhat developed when the rain water has been made faintly alkaline. Hence the stickiness depends upon the presence of a substance coagulable by heat, which acquires its adhesiveness through the action of the alkalinity and salts of sea water.

The coelomic fluid of Holothuria is practically sea water. Why, then, do not the tubes stick together as they lie in close contact in the body cavity? It has been generally observed that the tubes are adhesive only when extended. As a matter of fact, the stickiness increases in the slightly extended tubes after they have been for some minutes in seawater. But it would seem that in the usual rapid elongation of the organs the adhesive material is probably squeezed out of gland cells by the contraction of the wall of the tube— a conclusion which is favored by the structure of the organ.

V. SUMMARY

1. The Cuvierian organs of Holothuria captiva are expelled in response to mechanical stimulation, but not to various forms of chemical irritation.

2. The mechanism of their discharge comprises an intrinsic extending apparatus acting together with the stimulating action and directive influence of the cloacal current. The Cuvierian organs are not expelled by the forcing into them of water from the cloaca.

3. The adhesiveness of the discharged Cuvierian organs depends upon the presence of a heat-coagulable substance, which is altered in such a way as to become sticky when it is acted upon by the alkalinity and salts of sea water.

REFERENCES

CLARK, H. L. 1901. Bermudan echinoderms. Proc. Bost. Soc. Nat. Hist., vol. 29, no. 16, pp. 339–345.

CROZIER, W. J. 1914 a (?). The sensory reactions of Holothuria surinamensis Ludw. Zool. Jahrb., Abt. f. Physiol. (in press).

CROZIER, W. J. 1914 b. The orientation of a holothurian by light. Amer. Jour. Physiol., vol. 36, pp. 1–13, 6 figs.

DELAGE, Y., et HÉROUARD, E. 1903. Les échinodermes. Traité de Zool. concrète, T. 3, x + 495 p., 565 fig., 53 pls.

LUDWIG, H. 1892. Die Seewalzen. Bronn's Thier-Reich, Bd. 2, Abt. 3, Buch I, vi + 460 p., 25 figs., 17 pls.

MINCHIN, E. A. 1892. Notes on the Cuvierian organs of Holothuria nigra. Ann. Mag. Nat. Hist., Ser. 6, vol. 10, pp. 273–284, pl. 17.

MINES, G. R. 1912. Note on the mechanism of discharge of the Cuvierian organs of Holothuria nigra. Quart. Jour. Micr. Sci., vol. 57, pp. 301–304, pl. 26.

RUSSO, A. 1899. Sur valore morfologico e funzionale degli organi di Cuvier delle Oloturie. Monit. Zool. Ital., anno 10, pp. 225–228, 2 fig.

THE VASO-MOTOR NERVES OF THE DUODENUM

R. BURTON-OPITZ

From the Physiological Laboratory of Columbia University at the College of Physicians and Surgeons, New York

The arteria gastro-duodenalis which forms the continuation of the hepatic artery, gives rise to the arteria gastro-epiploica dextra and the arteria pancreatico-duodenalis. The former supplies the uppermost portion of the duodenum and adjoining pyloric end of the stomach, while the latter is directed towards the head of the pancreas, where it anastomoses with the arteria pancreatico-duodenalis inferior. During its course along the border of the middle segment of the duodenum this blood vessel gives off a number of branches which ascend upon both walls of this part of the intestine.

The nerve fibres originally embraced in the hepatic plexus, reach these parts by following the highways of the arteries. The plexus gastro-duodenalis divides into two networks at the point of bifurcation of the artery. A part of it then invades the pyloric region and another the pancreatic tissue, but besides, a number of filaments become separated from the main plexus and accompany the arterial twigs to the central segment of the duodenum.

In accordance with its blood and nerve supply the duodenum of the dog may be divided into three parts, namely, into an upper, a middle and a lower segment. While this peculiar anatomical arrangement has enabled me to prove the existence of vaso-motor nerves in the region of the pylorus[1] as well as in the lowermost portion of the duodenum,[2] it has been quite impossible so far to furnish similar proof for the central extent of this part of the intestine. This failure is to be attributed to the difficulties

[1] Burton-Opitz: Pflüger's Archiv, cxlvi, 1912, 344.
[2] Burton-Opitz: Pflüger's Archiv, cxxiv, 1908, 469.

encountered when a separation between the pancreatic tissue and the duodenum is attempted. To be sure, the blood supply of a large part of the pancreas can be determined satisfactorily, because this organ can be isolated from the intestine by simply ligating the duodenal branches of the pancreatic artery. In spite of the fact that the area of contact between the organs in question is not equally large in all animals; the reverse procedure meets with many obstacles, because the removal of the pancreatic tissue is generally attended by complete obliteration of many of the delicate duodenal blood vessels.

The animal from which the present data have been derived, presented rather favorable conditions in so far as the pancreatic artery pursued a very superficial course, so that its duodenal ramifications could be freed without serious injury to them and the accompanying nerve fibres. Having ligated the arteria gastro-epiploica dextra, the vascular field was restricted still further by obstructing all pancreatic branches. A stromuhr[3] was then inserted in the arteria gastro-duodenalis. The central cannula of this instrument gave attachment to the connecting tube of a membrane manometer for the determination of the pressure existing in this blood vessel. The distal end of the divided plexus gastro-duodenalis was placed in shielded electrodes at a point opposite the stromuhr.

This procedure enabled me to measure the quantity of blood distributed to the middle portion of the duodenum, while stimulation of the aforesaid plexus was resorted to at intervals. But, as it is extremely probable that the ligation of the different branches of the pancreatic artery markedly enhanced the flow through the duodenal channels, the quantitative data here given should be regarded merely as differential values.

In view of the present experimental arrangement, it must be clear that any variation in the blood supply of this part coincident with the excitation of this plexus, must be produced by active changes in the size of the duodenal blood-bed. To begin with the stromuhr registered a flow of 0.32 cc. in a second which, upon

[3] I employed the recording stromuhr described by me in Pflüger's Archiv, cxxi, 1908, 150.

Blood Supply of Duodenum

stimulation of the gastro-duodenal plexus with a moderate current, slowly decreased to 0.13 cc. in a second. The blood pressure which previously amounted to only 68.5 mm. Hg, rose gradually to 79.0 mm. Hg. Normal vascular conditions were again established soon after the cessation of the stimulation.

The curve reproduced in the accompanying plate shows the record of the stromuhr (S) in exact relation with the record of the membrane manometer (P) above the common abscissa E. The stimulation of the plexus gastro-duodenalis took place between points A and B. It is evident that the blood flow is considerably lessened during the seventh phase of the stromuhr, the decrease becoming well marked within a few seconds after the beginning of the stimulation. Between points C and D the flow attains its lowest value, while the arterial pressure rises to its highest level The excitation having been discontinued at B the flow and the pressure show a gradual return to normal at D.

These results which unmistakably point towards an active peripheral obstruction of the blood flow, were obtained several times in the course of this experiment. As the vascular field had been restricted in this animal to the central duodenum, it must be concluded that the blood vessels of this organ are innervated by nerves which ascend from the coeliac ganglion by way of the plexus gastro-duodenalis and plexus pancreatico-duodenalis.

THE BODY SURFACE OF FLOUNDERS AND ITS RELATION TO THE GASEOUS METABOLISM

SERGIUS MORGULIS

From the Woods Holl Laboratory of the Bureau of Fisheries

Received for publication November 21, 1914

It is an obvious fact that the surface of every organ bears an important relation to its function, and nature, by employing various means, has given to small masses surfaces a thousand times or more greater than would follow from their geometrical form. One thinks, naturally, of the lung, occupying a relatively small volume of the chest, but which has an exposure to the air representing an area of several thousand square meters, thanks to innumerable branches of the bronchi ending in minute alveoli. The blood which receives oxygen in the lung and distributes it to the various tissues is assisted in this office to an amazing degree by the great number of minute elements carrying oxygen, the red blood corpuscles, whose composite surface is very great as compared to their total mass. Such examples could be easily multiplied but even those already referred to suffice to point out the physiological significance of surfaces.

The outside surface of the entire organism, on the contrary, tends to approach as nearly as possible the geometrical minimum, apparently to reduce the area of contact with the surrounding medium and thus prevent unnecessary loss of heat. The exposure must, therefore, be comparable for all organisms in so far as it represents the smallest surface compatible with their mass. It is hereby assumed that the specific gravity is the same, which may be the case under similar physiological conditions. It must likewise be specified that this law of the minimum exposure may be counteracted by some special provisions for preventing the loss of heat, such as thickness of the skin or the nature of its covering.

The measurement of surface has been rarely undertaken because of its many difficulties. In the case of the higher mammals the prevailing methods are so cumbersome as to discourage their extensive use. As the consideration of surface relations, however, has attained much importance in the physiology of metabolism, a careful investigation seemed to me highly desirable. For this purpose I availed myself of the flounder, whose flat body can be easily and accurately measured. The method was extremely simple. The flounders were killed by pithing and weighed after the last traces of adherent water were removed. They were then laid flat on a stiff sheet of paper with the fins fully spread out, and an outline of the body was traced with a sharp pencil. The fins were then trimmed off close to the body, and an outline was again made of the finless animal. The weight was again determined, and a third outline made of the upper part of the specimen, which generally has a slight curvature. The area of this part of the flounder could be traced easily by rotating the flounder on its long axis. The surface was gotten from these three outlines which were measured by means of a planimeter. The maximum length, width and thickness of each specimen were likewise noted.

If the specific gravity of the body were one—in other words, if its mass and volume were equal, the surface could be expressed by the simple formula $S = \sqrt[3]{W^2}$ where W is the weight. As a matter of fact the surface is invariably greater than this, and under normal conditions is expressed by the formula $S = K\sqrt[3]{W^2}$, where S is the surface and K a constant factor. In metabolism studies this factor K determined for some particular animal has been applied to a wide range of physiological conditions. It will be shown in this report that results obtained in this way are highly unreliable.

By actually measuring the total surface of a large number of flounders, I computed the value of the factor K from the above formula. A glance at the subjoined table 1 will convince one that a very definite relation exists between surface and weight. The value of K ranges from 12.00 to 14.87 and from 7.82 to 9.59 for flounders with and without fins respectively. An examination

of the column of constants reveals that as the weight of the flounders increases the value of K tends to be smaller Averaging all the recorded values of K for flounders less than 10 cm. long, we find that it is 13.48 and 8.67 respectively; for flounders of over 10 cm. in length the average value of K is 13.37 and 8.25 respectively. For the entire lot of about forty specimens the average is 13.44 (or 8.5 exclusive of the fins).

The slightly higher constant for small flounders may perhaps suggest that they have a proportionately larger surface, but I am strongly convinced that it is due to the fact that they are frequently more poorly nourished than their more successful competitors for food, the larger flounders. At any rate, fasting, as will be shown later, invariably causes a very considerable increase in the value of K.

An examination of the data pertaining to the other measurements of each flounder shows some very interesting things. It will be observed that the width and especially the thickness change very slowly in comparison with the weight, but the length (from the tip of the snout to the base of the caudal fin) varies directly with it. Thus, with the maximum thickness increasing threefold—0.65 to 1.95 cm.—the body weight changed from 5.7 gram (average) to 148.2 grams, or 26 times. For a similar increase in width—from 1.8 to 5.4 cm.—the body weight changed from 1.45 grams to 43.5 grams, or 30 times. In both instances, however, the length changed from 6.85 to 19.80 cm. and from 4.6 to 13.0 cm. respectively; i.e., in either case practically in the same ratio as the weights (1 ÷ 3). The close relation between the weight and length is of much importance, as it affords a means of estimating the weight when the length is known, and vice versa.

The weight of the fins forms a comparatively small portion of the total mass of the fish, as will be seen by the figures of the third column. It will be noticed also that as the flounder grows bigger and heavier the fins become relatively lighter. The surface of the fins, however, represents 30–40 per cent of the total body surface. It is, therefore, possible to cause a considerable reduction of surface without appreciably affecting the weight

of the fish by cutting off the fins. If the rate of metabolism depended upon the surface, as some believe, it would be natural to expect a drop in the oxygen consumption concomitant with the removal of the fins. Experiments bearing on this question are described below.

A series of experiments were performed with three small flounders for the purpose of finding out the rate of oxygen consumption with a normal and reduced body surface. The results recorded in the first part of the table below show a considerable range of variation depending upon the state of nutrition and, especially, the behavior. After one week's experimentation all the fins were removed in two specimens, while in the third— the smallest— only the left fin was removed. The animals were apparently little discomforted by this operation, swam about at times quite actively and ate the food. Several experiments were then performed under conditions similar to those of the previous set. It will be noticed from the results of the second part of the table that the oxygen consumption per gram-hour is not different from what it had been before the fins were removed (except when the animals have been particularly restless) and the surface thereby diminished.

As the second part of the experiment in the foregoing series was performed several days after the operation a few other experiments were made to determine the more immediate effect of removing the fins. A flounder weighing 7.65 grams was used. In a preliminary experiment it was found that its oxygen consumption per gram-hour was 0.179 cc. At the close of the experiment all fins were removed and about an hour later a new experiment performed under precisely the same conditions. The oxygen consumption per gram-hour was 0.172 cc. this time. This flounder died the next day.

In the case of another flounder weighing 9.58 grams the oxygen consumption before and after the operation was 0.177 cc. and 0.154 cc. per gram-hour respectively. In either case the diminution in weight occasioned by the removal of the fins was insignificant (2.5–3.1 per cent), but the surface was reduced by fully one-third.

TABLE 1

WEIGHT IN GRAMS	WEIGHT IN GRAMS (WITHOUT FINS)	WEIGHT OF FINS IN PER CENT OF THE TOTAL WEIGHT	BODY MEASUREMENTS IN CM.				$S = K\sqrt[3]{W^2}$		SURFACE OF FINS IN PER CENT OF TOTAL SURFACE	VALUE OF K FOR TOTAL SURFACE	VALUE OF K FOR BODY SURFACE EXCLUSIVE OF FINS
			Total length	Same without caudal fin	Width	Thickness	Total body surface in sq. cm.	Same, exclusive of the fins			
0.695	0.655	5.8	4.3	3.7	1.3		10.54	7.22	33.2	13.45	9.58
0.755	0.710	6.0	4.5	3.7	1.4		12.33	7.33	40.5	14.87	9.21
0.845	0.790	6.5	4.6	3.9	1.4		12.96	8.20	36.8	14.56	9.59
1.045	0.975	6.7	4.9	4.0	1.6		14.05	9.21	34.5	13.64	9.36
1.300	1.220	6.2	5.2	4.3	1.6		14.30	9.62	32.7	12.00	8.42
1.445	1.340	7.3	5.5	4.6	1.8		17.57	11.19	36.4	13.75	9.21
1.730	1.640	5.2	5.8	4.8	1.9		19.28	12.68	34.2	13.40	9.10
1.840	1.740	5.4	5.7	4.6	1.85		19.38	11.88	38.7	12.90	8.21
1.870	1.820	?	5.8	4.8	2.0		19.56	12.96	?	12.88	8.69
2.340	2.230	4.5	6.3	5.2	?.1		22.70	14.76	35.0	12.88	8.64
2.610	2.475	5.2	6.5	5.45	2.15		24.94	15.31	38.7	13.16	8.37
2.630	2.480	5.7	6.45	5.25	2.10	0.50	26.00	15.78	39.3	13.64	8.62
3.075	2.880	6.3	6.6	5.5	2.10		26.76	16.24	39.3	12.65	8.02
3.090	2.980	3.6	6.7	5.6	2.30		26.92	18.00	33.1	12.69	8.69
3.490	3.260	6.6	6.9	5.7	2.35		29.63	18.75	36.7	12.88	8.53
4.035	3.860	4.3	7.5	6.2	2.35	0.55	35.58	21.30	40.1	14.04	8.66
4.300	4.120	4.2	7.5	6.2	2.40	0.65	34.76	21.30	38.7	13.15	8.29
4.690	4.480	4.5	7.8	6.55	2.55		41.04	24.24	40.9	14.65	8.92
5.750	5.520	4.0	8.3	6.85	2.60	0.65	42.11	26.11	38.0	13.12	8.36
5.800	5.560	4.1	8.0	6.75	2.70	0.65	40.88	25.20	38.3	12.67	8.03
6.110	5.870	4.0	8.5	7.00	2.70	0.65	44.13	26.71	39.5	13.20	8.21
7.740	7.350	5.0	9.1	7.40	3.05	0.70	53.94	31.68	41.3	13.79	8.38
8.280	7.940	4.1	9.5	7.80	3.15	0.75	54.30	34.56	36.4	13.27	8.68
11.180	10.67	4.6	10.6	8.50	3.40	0.75	73.23	41.85	42.9	14.65	8.63
13.13	12.60	4.0	10.5	8.60	3.70	0.85	75.90	46.25	39.0	13.64	8.55
15.35	14.88	3.1	11.4	9.40	3.65	0.95	80.80	48.32	40.2	13.08	8.00
15.50	14.91	3.8	11.55	9.35	3.65	0.95	85.33	50.09	41.3	13.72	8.28
22.80*	22.00	3.5	12.95	10.55	4.15	1.05	103.57	61.37	40.8	12.88	7.82
25.57	24.78	3.1	14.00	11.50	4.50	1.05	119.22	71.96	39.7	13.73	8.47
26.50	25.65	3.2	13.90	11.30	4.50	1.05	124.08	72.97	41.2	13.95	8.39
28.45	27.47	3.5	14.10	11.60	4.50	1.10	124.76	74.58	40.2	13.38	8.19
42.64	40.73	4.5	15.70	12.80	5.10	1.15	155.70	95.09	38.9	12.75	8.03
43.50	41.95	3.6	15.90	13.00	5.40	1.25	165.15	100.39	39.2	13.35	8.32
49.56	47.78	3.6	16.80	13.60	5.70	1.30	186.11	109.19	41.3	13.79	8.29
52.85	50.83	3.8	16.90	13.90	5.50	1.35	183.06	109.20	40.3	13.00	7.96
90.39	86.53	4.3	20.70	16.70	6.20	1.65	277.54	157.72	42.5	13.78	8.06
90.87	87.44	3.8	20.60	16.60	6.20	1.65	265.82	156.10	41.3	13.15	8.00
148.20	142.87	3.6	24.60	19.80	7.90	1.95	364.93	221.83	39.2	12.32	8.12

* Ripe female.

TABLE 2

DATE	BODY WEIGHT OF FLOUNDER			OXYGEN USED PER GRAM-HOUR BY FLOUNDER			REMARKS
	A	B	C	A	B	C	
	gm.	*gm.*	*gm.*	*cc.*	*cc.*	*cc.*	
VII. 4	6.99			0.182			24 hours after feeding.
VII. 5	6.84	4.15	1.62	0.162	0.186	0.128	24 hours fasting (A, 48 hours). A and B somewhat restless.
VII. 6	7.59	4.12	1.68	0.240	0.137	0.154	14 hours after feeding. A very restless.
VII. 7	7.52	3.97	1.60	0.160	0.160	0.157	Not fed. B somewhat restless.
VII. 8	7.34	3.98	1.66	0.202	0.143	0.181	Only C took food. A restless.
VII. 10	7.89	4.08	1.72	0.198	0.192	0.241	Fed liberally since July 8.
VII. 13	7.46	3.76	1.63	0.205	0.252	0.193	24 hours after feeding A and B very restless.
VII. 14	7.27	3.75	1.58	0.253	0.264	0.191	A and B very restless! Not fed yesterday.
VII. 18	7.20	3.44	1.72	0.171	0.174	0.221	Fed freely several days. Last feeding about 24 hours before experiment.
VII. 19	7.31	3.34	1.66	0.192	0.185	0.209	Were not fed yesterday. A slightly restless.
VII. 23	7.32	3.45	1.86	0.192	0.196	0.268	Fed regularly till yesterday. Fins have regenerated appreciably.

Still another experiment was performed with a large flounder weighing 49.58 grams. Before the operation its oxygen consumption per gram-hour was 0.101 cc., but after the operation 0.116 cc. This increase is rather noteworthy, especially since owing to the loss of the fins the flounder kept exceedingly quiet during the second part of the experiment. I am strongly inclined to believe that the strong contraction of the muscles of the body which immediately resulted from the removal of the fins is responsible for this increased demand for oxygen. During the following couple of days the oxygen consumption dropped very considerably, but the animal was sickly and soon died. More experiments with large flounders will, therefore, be necessary to determine the effect of the removal of the fins.

Under Rubner's influence the general belief prevails that the gaseous metabolism is proportional to the body surface, and that

per unit of area it is the same for all animals. The theoretical basis for this generalization is the simple physical principle of radiation of heat which is proportional to the exposed surface. Since the metabolic processes are associated with the production of heat, the latter depending largely upon the rate of its dissipation to the outside environment, the metabolic processes must bear a direct relation to the surface. The figures generally given in support of this generalization cannot, without a stretch of the imagination, form a solid foundation for it. Pütter,[1] indeed, is very emphatic in pointing out the great variability of these figures and in his contention that the body surface has no relation to metabolic processes. But the lack of actual measurements of surface, above everything else, deprives Rubner's theory of its scientific value.

In the table that follows data which have been gathered at different times are brought together. These throw an entirely new light on this problem. The weight, surface and oxygen requirements have been actually measured. In a few instances the surface was not measured at the same time when the weight was determined. In these cases both the actually found surface and the theoretically expected surface are recorded. The difference between these two is of little significance.

Weight of flounder in grams	0.70	1.63	2.83	4.02	4.04	5.29	6.99	7.65	9.58	24.0	44.5
Oxygen consumption per hour in cc.	0.170	0.314	0.523	0.662	0.762	1.038	1.270	1.370	1.690	4.08	6.25
Oxygen consumption per gram-hour in cc.	0.241	0.193	0.185	0.155	0.189	0.196	0.182	0.179	0.177	0.170	0.140
Surface of flounder in sq. cm.	11.41 (10.49)	18.42	26.21	33.63	34.76 (33.74)	38.82 (40.38)	48.62	51.64	59.99	109.00	162.18
Oxygen consumption per sq. cm.-hour in cc.	0.015 (0.017)	0.017	0.020	0.020	0.022 (0.023	0.027 (0.026)	0.026	0.026	0.028	0.037	0.038

It must be inferred from these results that, while per unit of body weight the oxygen consumption diminishes as the size

[1] Pütter, August, 1911. Active Oberfläche und Organfunktion, Zeitschr. f. allg. Physiol., 12, 125–214.

of the flounder increases, the oxygen consumption per unit of body surface increases very regularly. In other words, the larger the flounder the larger its oxygen requirements per square cm. This very obviously results from the direct dependence of the metabolic exchange upon the mass of the organism which grows more rapidly than the surface.

The value of K is nearly constant so long as the physiological conditions remain the same. We are, therefore, quite unjustified in computing the surface of a fasting organism, for instance, from the formula $S = K\sqrt[3]{W^2}$ as is the common practice, using the value K established for a normal individual. There are two reasons for this: first, the body surface does not diminish in the same ratio as the body weight; second, the specific gravity is lower than normal owing to the greater content of water in the fasting organism. Measurements of surface reveal that these two factors conspire to raise the value of K. In the following table are given the results obtained from the study of several flounders which have been deprived of food for 5 to 28 days. At the close of the fasting experiment both the weight and surface of each specimen were carefully measured. Comparing these data with those recorded in the first table it is seen that the area of the organism has changed only to a very slight degree. It will also be of interest to observe that the relative weight and the proportion of the area covered by the fins at the end of a long fasting period remains practically the same as before fasting.

DAYS OF FASTING	INITIAL WEIGHT	FINAL WEIGHT		RELATIVE WEIGHT OF FINS	BODY DIMENSIONS				INITIAL SURFACE (estimated)			FINAL SURFACE		RELATIVE SURFACE OF FINS	VALUE OF K	
		Total	Without fins		Total length	Length (to caudal fin)	Width	Thickness	Value of K (computed)	Surface	Surface (K=13.44)	Total	Same without fins		Total surface	Same exclusive of fins
	grams			*per cent*	*cm.*									*per cent*		
22	0.705	0.415	0.375	9.6	4.25	3.50	1.35	0.20	14.19	11.41	10.32	11.24	6.88	38.8	20 21	13.23
15	3.30	2.165	2.06	4.9	6.9	5.7	2.15		12.86	29.17	29.48	28.60	17.46	39.0	17.09	10.78
28	4.04	2.69	2.55	5.2					13.50	34.76	33.74	34.25	19.99	41.6	17.71	10.58
28	5.29	3.47	3.32	4.3	7.75	6.40	2.65	0.45	13.60	38.82	40.38	38.25	23.07	39.7	16.72	10.37
5	44.5	41.96	40.36	3.8	16.1	13.6	5.4	1.20	12.40	162.18	167.02	159.78	101.1	36.8	13.22	8.59

The value of K, which for normal flounders has been found to be 13.44, varies from 17.09 to 20.21 and from 10.37 to 13.23 for flounders with and without the fins respectively. (The case of the large flounder where the fast was too short to induce a noticeable change is neglected.) The initial surface is estimated by two methods. First, the value of K is computed from the formula $S = \sqrt[3]{W_1^2}$, where W_1 – the initial weight – is substituted for W – the final weight. From the average of these constants the deviation from the normal is determined, which in this case is about 2.5 per cent, and a corresponding correction is introduced in the total surface. The second method consists in estimating the initial surface with the aid of the average constant ($K = 13.44$) which has been established for normal flounders.

If one compares the oxygen consumption of the first four flounders at the beginning and end of the fast the interesting thing appears that per square cm. the oxygen decreases in every instance to from one-third to two-thirds of the original amount. If, however, the final body surface—which in this experiment has been actually measured—were estimated from the final weight with the aid of the constant 13.44, as is generally practiced, it would be found that at times the oxygen consumption per square cm. remains unchanged. This is shown in the table below:

INITIAL SURFACE IN SQ. CM.	OXYGEN IN CC. PER SQ. CM. AND HOUR	FINAL SURFACE (MEASURED)	OXYGEN IN CC. PER SQ. CM. AND HOUR	FINAL SURFACE (COMPUTED WITH FACTOR 13.44)	OXYGEN IN CC. PER SQ. CM. AND HOUR
11.41	0.015	11.24	0.010	7.47	0.015
29.17	0.026	28.60	0.019	22.44	0.024
34.76	0.022	34.25	0.011	31.67	0.012
38.82	0.027	38.25	0.008	30.84	0.011

It is obvious, therefore, that a scientifically unqualified trust in mere computation is probably responsible for the broad generalization according to which the metabolic processes are the same when referred to the unit of surface. The coincidence of the data for the oxygen consumption per square cm. in the

first two cases may serve as a warning against conclusions which have been drawn without any foundation or reference to the facts.

The following conclusions may be drawn from the above investigation. The surface of flounders can be computed by the ordinary formula $S = K\sqrt[3]{W^2}$, whereby the average value of the constant factor K for *normal* flounders has been found to be 13.44. This coincides very closely with the value which has been found for higher organisms.

The metabolic processes of the flounder bear direct relation neither to the weight nor surface of the flounder, the oxygen consumption per gram-hour diminishing but increasing per square centimeter-hour as the flounders become larger.

Removal of the fins, causing a reduction of surface of over 30 per cent without materially changing the mass of the flounders, does not affect the oxygen consumption, provided no disturbing factors are present. Under these conditions the oxygen consumption is proportional to the mass.

The value of K changes according to the physiological state of the organism. For fasting flounders, owing to the fact that the surface does not diminish in the same ratio as the mass, and the specific gravity of the body also decreases, the value of K is considerably higher than normal and must be determined in each individual case.

THE INFLUENCE OF PREGNANCY ON THE HYPERGLYCEMIA OF PANCREATIC DIABETES

A. J. CARLSON AND H. GINSBURG
From the Hull Physiological Laboratory of the University of Chicago

Received for publication November 24, 1914

It has been shown that late pregnancy prevents the characteristic onset of severe diabetes following complete pancreatectomy. To account for this, the suggestion was made that in pregnancy near term the internal secretion from the fetal pancreas passes into the maternal blood in sufficient amount to maintain the normal carbohydrtate metabolism.[1]

In the previous work the presence or absence of diabetes was determined by urine examination alone. There is a mere possibility that late pregnancy in dogs may have some injurious action on the kidneys so as to diminish or prevent the excretion of sugar, in which case we might have the hyperglycemia of diabetes without glycosuria. This possibility has now been tested on four pregnant dogs near term, and we desire to place on record a summary of our results in the present report.

EXPERIMENTAL PROCEDURE

1. Blood Sugar

The blood sugar was determined by the Rona-Michaelis method.

2. Drawing Blood

The blood (about 5 cc.) was drawn either from the saphenous vein by a hypodermic needle, or from the tail. If sufficient care is taken there is no pain or struggling to cause temporary hyperglycemia, even when no local anaesthesia is employed.

[1] Carlson and Drennan: This Journal, 1911, xxviii, p. 391; Carlson, Orr and Jones: Jour. of Biol. Chem., 1914, xviii, p. 19.

In every case a sample of normal blood was drawn before the pancreatectomy and at varying intervals after the operation until the pups were born. The animal was watched as closely as possible night and day so that samples of blood could be drawn at the onset of labor and also after parturition.

RESULTS

We succeeded in getting only ten suitable dogs, as the pregnant bitches near term are not readily obtained. Complete pancreatectomy was made in all of these dogs, but six yielded no results, as abortion occurred within seven to twenty-one hours after the operation.

Pregnant Bitch I

The normal blood sugar content in this animal before pancreatectomy was 0.08 per cent. The following eight hours after the operation there was a marked hyperglycemia. This is probably post-anaesthetic and post-operative hyperglycemia. Twelve hours later the blood sugar returned to its normal level and remained there for the subsequent thirty-one hours. The dog remained in good spirits. When labor set in there was an abrupt

TABLE I

Pregnant Bitch I. Complete pancreatectomy May 28, 1914. Recovery good. No infection

HOUR SAMPLE TAKEN	BLOOD SUGAR
	per cent
Normal	0.08
3 hours after pancreatectomy	0.111
8½ hours after pancreatectomy	0.174
12 hours after pancreatectomy	0.090
15 hours after pancreatectomy	0.090
19 hours after pancreatectomy	0.090
24 hours after pancreatectomy	0.070
31[1] hours after pancreatectomy	0.113
40[2] hours after pancreatectomy	0.200

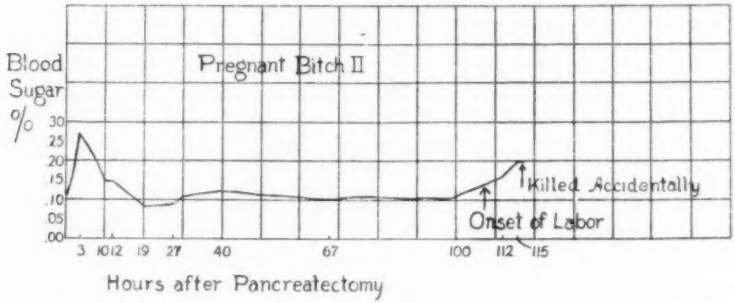
[1] Dog in labor, one pup born.

[2] Five pups born, experiment discontinued.

rise in the blood sugar to the high level (0.20 per cent) present in experimental pancreatic diabetes and in the typical clinical condition of "severe diabetes" in man. The fact that the blood sugar rose and remained at this level indicated that the pancreatectomy was complete. This was verified at necropsy.

Pregnant Bitch II

After the operation this dog was given a daily diet of 150 cc. goat's milk, 100 grams of lean meat, and plenty of water. Following the initial post-operative hyperglycemia this dog ran a typical normal blood sugar curve up to noon of the fifth day



(112 hours) when the dog went into labor. A blood sample taken then showed 0.15 per cent sugar. Another sample taken three hours later showed 0.20 per cent. As the animal was weak and the uterine contractions apparently very feeble, we feared that she would not be capable of delivering the pups, and decided to perform a Caesarean section, but, unfortunately, while preparing for this operation, the bitch was accidentally killed. At autopsy one pup was found engaged in the vagina and nine others were found in utero, of these five were still living. The pups were practically at full term. The duodenum was carefully examined but no trace of pancreatic tissue was found.

TABLE II

Pregnant Bitch II. Complete pancreatectomy June 19, 1914. Recovery good. No infection

HOUR SAMPLE TAKEN	BLOOD SUGAR
	per cent
Normal	0.100
3 hours after pancreatectomy	0.270
10 hours after pancreatectomy	0.150
12 hours after pancreatectomy	0.147
19 hours after pancreatectomy	0.087
27 hours after pancreatectomy	0.105
40 hours after pancreatectomy	0.123
67 hours after pancreatectomy	0.101
100 hours after pancreatectomy	0.119
112 hours[1] after pancreatectomy	0.150
115 hours[2] after pancreatectomy	0.200

[1] Dog in labor.

[2] Dog accidentally killed; 10 pups in utero.

Pregnant Bitch III

This dog retained her fetuses thirty-one hours, when abortion occurred and three premature pups were delivered. The sample of blood taken just before abortion revealed a blood sugar content of 0.23 per cent, showing that the break between the uterus and the pups had already occurred. The fact that the blood sugar

TABLE III

Pregnant Bitch III. Pancreatectomy July 14, 1914. No infection

HOUR SAMPLE TAKEN	BLOOD SUGAR
	per cent
Normal	0.097
3 hours after pancreatectomy	0.160
9 hours after pancreatectomy	0.160
12 hours after pancreatectomy	0.155
17 hours after pancreatectomy	0.160
25 hours after pancreatectomy	0.159
31[1] hours after pancreatectomy	0.230
43 hours after pancreatectomy	0.400

[1] Three pups born.

never completely returned to its normal level would seem to be due to lack of sufficient pancreatic tissue in the three pups, especially as these pups were premature (about three weeks of term). At subsequent autopsy no pancreatic tissue was found in the mother.

Pregnant Bitch IV

This dog retained the fetuses two full days, and except for a temporary post-operative rise the blood sugar was slightly above normal throughout. There was no sugar in the urine.

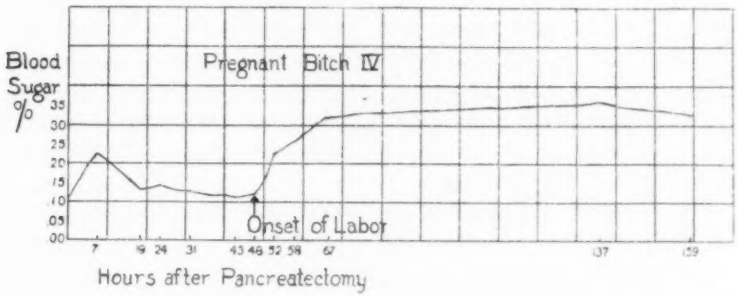


TABLE IV

Pregnant Bitch IV. Pancreatectomy July 31, 1914. Good recovery. No infection

HOUR SAMPLE TAKEN	BLOOD SUGAR
	per cent
Normal	0.110
7 hours after pancreatectomy	0.200
19 hours after pancreatectomy	0.130
24 hours after pancreatectomy	0.140
31 hours after pancreatectomy	0.119
43 hours after pancreatectomy	0.114
48 hours[1] after pancreatectomy	0.113
52 hours[2] after pancreatectomy	0.220
58 hours after pancreatectomy	0.260
67 hours after pancreatectomy	0.320
137 hours after pancreatectomy	0.360
159 hours after pancreatectomy	0.330

[1] Two pups born.
[2] Six pups born.

Two pups were born at the end of the forty-eight hours, but the blood sugar was not affected in the least, as there were still six fetuses in the uterus. When these fetuses severed their connection with the maternal organism four hours later, the blood sugar rose immediately. No pancreatic tissue was found at autopsy.

SUMMARY

Total extirpation of the pancreas in non-pregnant dogs results in the onset of pancreatic diabetes within seven to twelve hours. Complete pancreatectomy in pregnant bitches near term is not followed by hyperglycemia and glycosuria, etc., as long as the fetuses are alive and the placental connections are not severed. At the onset of labor the blood sugar begins to rise, so that the hyperglycemia with consequent glycosuria, characteristic of pancreatic as well as severe clinical diabetes is established on the completion of the delivery.

This absence of diabetes may be due either to a secretion from the fetal pancreas reaching the maternal blood, or to some detoxicating action on the part of the fetal pancreas. We hope to definitely prove or disprove the first possibility by transfusion experiments now in progress.

ON THE VALIDITY OF INDUCTORIUM CALIBRATIONS

E. G. MARTIN

From the Laboratory of Physiology in the Harvard Medical School

Received for publication November 30, 1914

As the result of an extended study, with the aid of the string galvanometer, of induction shocks generated under various conditions Erlanger and Garrey[1] have recently pointed out certain differences in rate of growth, amplitude, duration, or contained current that may be shown by induction shocks of equivalent physiological intensity. On the basis of these observed differences they are inclined to question the validity of the systems of inductorium calibration now in vogue. Inasmuch as I have developed and used one such system[2] I desire to consider its validity in the light of their observations.

Degree of accuracy required. When an induction shock traverses a mass of living tissue the current composing the shock distributes itself among the various conducting pathways of the tissue according to their relative resistances, in obedience to the law of divided circuits. Of these various conducting pathways many doubtless are non-irritable, so that the physiological effect of the shock is exerted only by that part of it which comes into direct contact with irritable tissue. This irritable tissue, moreover, is undoubtedly in a state of unstable electrolytic equilibrium, undergoing frequent variations in conductivity, especially as the tissue becomes active in response to the stimulus of the shock itself. Furthermore, the structure of most tissues, with non-conducting lipoid membranes distributed throughout the general conducting mass, is that of a system of condensers, so that we have to reckon with the phenomenon of capacity, and varying capacity, as well as with the other variables involved. In view

[1] Erlanger and Garrey: This Journal, xxxv, 1914, 377.

[2] Martin: The measurement of induction shocks, New York, 1912.

of these facts one may reasonably anticipate that shocks which may be perfectly superimposed physically will produce different physiological effects at different times in one and the same tissue. As a matter of fact such differences of effect do occur constantly. We speak of them as due to changes of irritability in the tissue, although in reality they are doubtless often the result of changes in the distribution of the currents among the different conducting pathways, irritable and non-irritable, therein. One may well wonder, not that tissues display variations in responsiveness, but that they ever remain so constant as they frequently do.

I have only fragmentary data concerning the extent of error which may result from the sources just mentioned. On the basis of such as I have my practice has been to assume that the error may often amount to 10 per cent of the initial value of the stimulus. Since these sources of error are inevitable in all physiological employment of induction shocks I take the view with regard to the quantitative use of such shocks, held generally by workers in science, that refinement of one portion of a procedure beyond the inherent error of other portions is not only unnecessary, but is likely to convey an impression of greater accuracy than actually exists. In my own researches I do not base conclusions on observed differences amounting to less than 10 per cent unless they occur so frequently under particular conditions as to afford assurance that they are not accidental. In the development of an inductorium calibration for physiological use, therefore, I feel that as a criterion for stimuli that are to be rated as physiologically equivalent, exact physical superimposability is excessive.

In 1908 I urged the importance of the physiological criterion as the proper basis for a physiological evaluation of induction shocks.[3] That position I still hold, and the particular question I wish to consider in this paper is whether the calibration I have developed satisfies reasonably the physiological criterion. The problem presents itself in two phases: (1) does the calibration afford valid basis for comparisons of relative stimulation strengths on any one coil, and (2) can valid comparisons be made between different coils?

[3] Martin: This Journal, xxii, 1908, 116.

The calibration curve as an expression of relative stimulation values. To express relative stimulation values for the various positions of the secondary coil with respect to the primary, resort has been had, in most of the systems thus far proposed, to curves based on determinations of the relative amounts of contained current in shocks generated at the different secondary coil positions, by the break of a fixed primary current. Erlanger and Garrey object to this method (p. 442) on the strength of observations in which they showed that approximating the coils modifies so profoundly the *contour* of the shocks that physiologically equivalent fractions include different amounts of contained current. In making these observations Erlanger and Garrey desired to avoid variations in the total resistance of the secondary circuit. They therefore used a low resistance shunt (32 ohms) across the terminals of their secondary coil, and led off from this through their test muscles threshold fractions. A necessary result of this arrangement was that to get fractional shocks of threshold magnitude the secondary coil had to be brought into the immediate region of the primary. In one coil they used positions 12.2 cm. and 0 cm.; in another 11.1 cm. and 0 cm. (p. 441). I pointed out in my first presentation of a calibration scheme[4] that in inductoria with iron cores, such as Erlanger and Garrey used, the relation of contained current to physiological efficiency holds for different secondary positions only over the *outer* parts of the field, regions beyond the influence of the iron core. That this relationship does hold over the outer parts of the field I have proved by a large number of experiments in which more than a dozen different inductoria have been used. The failure of agreement of contained current with physiological efficiency in the portions of the field where the secondary coil overlaps the primary, necessitates, as I showed formerly (loc. cit.), the abandonment of the physical criterion for this part of the scale, and the substitution for it of a physiological criterion. The one I used, the v. Fleischl test, was used also by Erlanger and Garrey in their investigation, and appears to be valid for all comparisons for which *threshold* stimuli

[4] Martin: Loc. cit., 121.

can be employed. By means of this physiological test I showed that a calibration can be worked out for the part of the field where the physical criterion fails, and that the calibration so established is sufficiently accurate for the needs of physiologists.

The influence of secondary resistance on the form of the calibration curve. Erlanger and Garrey point out (p. 445) that the effect of changing the secondary resistance is to alter the configuration of an induction shock, and also to modify its stimulating effectiveness; facts which have been noted likewise by earlier observers.[5] They offer the opinion that this effect of secondary resistance on configuration, taken in connection with the influence of approximating the secondary coil to the primary, and with possible other factors, still unknown, complicates the problem of evaluating faradic stimuli beyond present possibility of solution. I am inclined to agree with them, so far as purely physical means of evaluation are concerned. Nevertheless, while they are doubtless correct (save for the inherent variations noted above, p. 223) in their statement (loc. cit., p. 447) that shocks would be equal physiologically if they could be exactly superimposed physically, Erlanger and Garrey would doubtless admit the possibility of the existence of shocks, equivalent physiologically, which could not be superimposed physically. The working physiologist is interested in the physiological equivalence of shocks. The test as to whether they are thus equivalent must be, in the last analysis, a physiological test. Granting that the effectiveness of shocks is modified by variations in secondary resistance, the problem is, can a valid method be worked out for taking this modification into account?

In attacking such a problem one must consider not only the effect upon physiological intensity of changes of resistance when all other factors remain constant, but also the effect of such changes in connection with others occurring simultaneously. An example of the latter situation cited by Erlanger and Garrey, and by them urged as evidence against the validity of inductorium calibrations, is the case in which there is approximation of the secondary coil to the primary in connection with changes in sec-

[5] For references see Martin: This Journal, xxvii, 1910, 226; and xxviii, 1911, 49.

ondary resistance (p. 447). Although, as shown above, Erlanger and Garrey were in error in their view that approximating the secondary coil to the primary invariably disturbs the relationship between physical and physiological intensity, a disturbance which actually appears only in that restricted portion of the field comprehended within the length of the primary coil, it will perhaps be instructive to consider the question they raise with the aid of the physiological criterion.

If simultaneous changes in secondary resistance and secondary coil position affect the physiological efficiencies of shocks so profoundly as Erlanger and Garrey intimate, obviously a calibration scale for the various secondary position which holds for low resistances should not hold for high ones. To test this point I have examined a number of the experiments I performed during the period 1906–1910 in connection with the development of a calibration scheme. In these experiments the threshold stimulus was determined for various secondary positions by the v. Fleischl method. Frogs' gastrocnemius muscles and frogs' sciatic nerves were stimulated. The values of the stimuli were expressed in *Z* units.[6] In my experiments the intensity of the shocks was cut down to threshold dimensions by cutting down the primary current, instead of by using a shunt as did Erlanger and Garrey.

In these experiments three different inductoria were used, all of the general type recommended by me for quantitative work,[7] having secondary coils about 13 cm. long, with approximately 10,000 windings. I have collected the data from 104 experiments. In 43 the total secondary resistance was less than 10,000 ohms, ranging as low as 3,100 ohms; in 24 the secondary resistance fell between 10,000 and 20,000 ohms; in 11 between 20,000 and 30,000 ohms; and in 26 above 30,000 ohms. I will report, first, the data covering the *outer* part of the field of the inductorium, the region where, according to my observations,[8] the physiological effects of shocks are proportional to their "contained currents." In each experiment threshold determinations were made at several secondary positions In each of eight experiments

[6] Martin: The measurement of induction shocks, 73.

[7] Martin: Ibid., 89.

[8] Martin: This Journal, xxii, 1908, 121.

only three such determinations were made in the outer part of the field, namely, beyond 12 cm. In each of the remaining 96 experiments four or more determinations were made in this region. If the calibration scale is correct, and the irritability of the experimental tissue does not vary, the values of Z for any one experiment should be constant Approximate constancy over a large series of experiments suffices to establish the correctness of the calibration. In each experiment I determined the average Z from all the values, found experimentally, beyond 12 cm. Then the percentage divergence from this average of the most divergent single reading was calculated. Comparisons of the number and extent of these percentage divergences, under different conditions as regards secondary resistance, afford a means of judging fairly whether or no changes in secondary resistance affect the validity of the portion of the calibration scale now under consideration.

In the 43 experiments with low secondary resistance (less than 10,000 ohms) 36 showed no single reading diverging from its average as much as 7 per cent; 4 showed single divergences falling between 7 and 10 per cent; in each of the remaining three there were single divergences greater than 10, but less than 20, per cent. In the 24 experiments with secondary resistance between 10,000 and 20,000 ohms 21 showed no divergences exceeding 7 per cent, and in none of the experiments was there a divergence as great as 10 per cent. In the 11 experiments with secondary resistance between 20,000 and 30,000 ohms, 9 held constant within 7 per cent, and none showed divergence amounting to 10 per cent. In the 26 experiments with secondary resistance exceeding 30,000 ohms 19 showed no divergence above 7 per cent, and only 2 of the 26 showed divergences exceeding 10 per cent. Furthermore, any distortion of the calibration scale which might be brought about by changing the secondary resistance ought to show itself by similar divergences in experiments with similar secondary resistance. As a matter of fact the divergences which occurred were scattered irregularly over the whole field, and doubtless were due, for the most part, to errors in exact establishment of the threshold.

On the basis of the facts cited thus far I would offer the following conclusion: *A calibration scale, established for the outer part of the field of a "standard" inductorium, on the basis of the "contained current" in the shocks, expresses accurately the influence, on the physiological efficiency of the shocks, of shifting the secondary coil, no matter what the resistance in the secondary circuit.*

Turn now to the situation which exists in the inner portion of the field, that portion within the influence of the iron core. Here are realized conditions which, according to Erlanger and Garrey, tend to invalidate calibration scales, namely, the possible simultaneous occurrence of two distinct influences, shifts in secondary coil position and variations in secondary resistance, either of which affects shock contour independently of the other. It is quite true that in this part of the field a calibration scale that holds for low secondary resistances will not be wholly correct for high ones. This I pointed out in 1912.[9] The problem here is strictly a practical one. Recognizing the desirability of including this portion of the field so far as possible in a quantitative scheme, can a calibration be established in which the limits of error shall not be so great as to destroy its serviceability? On the ground that a calibration which is accurate within 15 per cent is useful, if its limitations are clearly understood, I have examined the same series of experiments as was discussed above, to learn to what extent the calibration worked out by me for the inner portion of the field departs from this degree of accuracy with different secondary resistances. The calibration was established originally by observations on tissues whose secondary resistances would rarely exceed 10,000 ohms. In the experiments with secondary resistances below this figure divergences from the average value of Z exceeding 15 per cent were relatively few, numbering only 17 out of 108 readings, the latter made at various points on the scale between positions 0 cm. and 8 cm. Of these 17 marked divergences, 11 were above the average and 6 below, showing that they were accidental, rather than indicative of an error in the calibration. The experiments in which resistances greater than 10,000 ohms occurred

[9] Martin: The measurements of induction shocks, 59.

can best be considered in two divisions; first with reference to the portion of the inductorium field from the outer end of the primary coil as far in as position 6 cm.; and second with reference to the very innermost portion of the field. For the first division readings at positions 8 cm. and 6 cm. were used exclusively, since a calibration that is shown to be valid for these positions must hold for regions beyond. Of 51 readings at positions 8 cm. and 6 cm., with secondary resistances above 10,000 ohms, 7 diverged from the average more than 15 per cent. Of these 7 divergences, 6 occurred in experiments in which the secondary resistance exceeded 30,000 ohms. All 7 were below their respective averages, as were also more than 70 per cent of the minor divergences. At secondary positions 4 cm. and 0 cm. the experiments included 82 readings in which secondary resistance exceeded 10,000 ohms. Of these 82, 33 were more than 15 per cent divergent. All these large divergences were below their respective averages, as were also more than 80 per cent of the minor divergences. Of the 33 large divergences of this series, 24 were from among the 40 cases in which the secondary resistance exceeded 20,000 ohms.

A just interpretation of the above data seems to me to be that *a calibration scale established for the inner portion of the inductorium field by experiments upon tissues of low resistance holds with less and less accuracy for high secondary resistances the closer the secondary coil is approximated to the position of complete superposition, and also the greater the resistance; but that for practical purposes the calibration is sufficiently accurate over the range of resistances likely to occur in physiological work as far in as position 6 cm. in a "standard" inductorium.* In accordance with this view my practice of late in calibrating inductoria has been to carry the calibration no further toward the zero position than 6 cm. The slight resultant diminution in range of stimulating power is readily compensated for by increasing the primary current.

The relation of secondary resistance to the physiological efficiency of faradic stimuli. The discussion presented in the above paragraphs has for its sole purpose the demonstration that a *cali-*

bration applied to a "standard" inductorium, in accordance with the scheme I have developed, is valid with reference to threshold stimuli generated in any tissue regardless of the resistance of the tissue. The further question, whether, in two different tissues of the same resistance, shocks identical according to the calibration, would necessarily be physiologically equivalent, requires special consideration. In a former paper[10] I have discussed this question exhaustively, and have shown that although stimuli for individual tissues must be dealt with individually, so that comparisons of stimuli imparted to one tissue with those imparted to another cannot be made directly, with a high degree of accuracy, yet in those cases in which threshold irritabilities can be determined, allowance can be made for the factors which tend to render direct comparisons inaccurate. I see no occasion for reviewing here the method by which this is done. I wish to say only that application of the method in hundreds of experiments has been fruitful of information not likely to have been afforded by procedures in which it was not employed.

The applicability of the calibration to stimuli above the threshold. Erlanger and Garrey (loc. cit., p. 442) question the applicability of a calibration based on threshold shocks to stimuli above the threshold. It happens that the observation which occasions their question is the incomplete one previously discussed of the effect on "contained current" and physiological efficiency of approximating the coils. That the disturbance of the relationship between these factors occurs only in the inner portion of the field has been pointed out above. Significant in this connection is the fact that the disturbance of relationship in this region applies fully to *threshold* shocks, so that in a calibration established for this region according to the method I have employed the indicated physiological efficiencies will not be proportional to the contained currents. While the raising of this question is thus seen not to be necessitated by any of the reported observations of Erlanger and Garrey, the problem may well be considered briefly on its own merits. The fundamental assumption on which rests the validity of the calibration for stimuli

[10] Martin: This Journal, xxvii, 1910, 226.

above the threshold is that *so long as other factors remain constant, the effectiveness of a stimulus is directly proportional to the intensity of the primary current whose break or make generates it.* Erlanger and Garrey report no galvanometric studies of the effects on induction shocks of varying the primary current, but such evidence as is available from other sources accords completely with the assumption. One such line of evidence is derived from a study of the data obtained in the preparation of a calibration scale for an inductorium. An early step in the procedure is the determination, in the outer part of the field, of the galvanometer deflections brought about at different secondary positions by the break of a *fixed* current through the primary. If a fixed current is not actually used the results are reduced to the values they would have if the current were fixed. These deflections are plotted in the form of a curve and are considered to represent the relative physiological efficiencies of the secondary positions at which they were obtained. To prove that they do truly bear this relationship the v. Fleischl test is applied. In this test the *primary current is adjusted* until a threshold shock is found. The curve of galvanometer deflections, based on a fixed primary current, is deemed correct if the products of the deflections at the various secondary positions by the primary currents required to generate threshold shocks at the same positions give a constant. As a matter of fact constants are obtained regularly under these conditions, although the irritabilities of the test tissues vary so greatly that the primary currents required in different experiments to generate threshold shocks at particular secondary positions will scarcely be found twice alike, and may vary over an enormous range. I find, for example, among the 104 experiments cited in earlier paragraphs of this paper, primary currents, used at position 12 cm. to elicit threshold shocks, ranging from 0.0003 ampere, to 0.1 ampere. If the physiological efficiencies of shocks were not directly proportional to the intensities of the primary currents used in generating them, constant results could not be obtained under these conditions.

A second line of evidence is afforded by experiments in which threshold fractions of shocks are obtained by means of shunts in the secondary circuit. Where shunts are used thus, the primary currents required to generate shocks of threshold intensity will differ according as the resistance of the shunt varies. We have here a means, therefore, of determining directly whether changes in the primary current bring about equivalent variations in physiological effect. In the use of this test the influence of secondary resistance on physiological effectiveness must be taken into account. This can readily be done by applying the principle upon which rests my system of correcting for secondary resistance, namely, that if the values of Z obtained experimentally by eliciting equivalent shocks with various secondary resistances, are plotted against the resistances, the resulting curve is a straight line, whose formula I have determined.[11] The actual data from a test of this character are as follows: Experiment of February 5, 1909. Values of Z (primary current times "calibration number") were determined at secondary coil positions 8 cm., 12 cm., 18 cm., and 24 cm., for the threshold of a frog's sciatic-gastrocnemius preparation under three conditions of secondary resistance; (1) with the tissue only (res. 4900 ohms) in circuit with the secondary coil (res. 1400 ohms); (2) with 6000 ohms added resistance in the secondary circuit; (3) with a shunt of 2000 ohms resistance in the secondary circuit. With each of these resistances closely concordant values of Z were obtained for the four secondary positions at which tests were made. Their average values, together with the total secondary resistances, were: for (1) $Z = 2.73$, total secondary resistance = 6300 ohms; for (2) $Z = 3.45$, total secondary resistance = 10,300 ohms; for (3) $Z = 7.4$, total secondary resistance = 2800 ohms. If to the figures of (1) and (2) the Wilbur modification[12] of my equations for correcting for secondary resistance, $\beta = \frac{Z_R R' - Z_{R'} R}{R' - R}$, be applied, the value of β for this particular preparation can be calculated, and, in turn, from this

[11] Martin: Loc. cit., 228.

[12] Martin, Bigelow and Wilbur: This Journal, xxxiii, 1914, 416.

value and the data of (1) the theoretical value of Z for any other secondary resistance can be determined. By applying these calculations, the theoretical value of Z for a secondary resistance of 2800 ohms (the resistance of (3)) is found to be 2.10. The experimental value of Z for (3), 7.4, represents a shock that divided itself between the tissue (res. 4900 ohms) and the shunt (res. 2000 ohms). If from the equation for the flow of currents through divided circuits we compute the fractional part of Z (3) that passed through the tissue, it is found to have a value of 2.15. This agrees with the theoretical value 2.10, determined above, well within the limit of error. Repetition of this experiment with other secondary resistances and other shunt resistances have given equally concordant results. In this experiment primary currents of greater than threshold intensity were used. No impairment of the relationships expressed by the calibration was manifest. We may conclude, on the basis of these two lines of evidence, that the stimulating effectiveness of induction shocks is directly proportional to the intensity of the primary currents used in generating them. If this conclusion is correct the assumption is equally justified that the relationships expressed by an inductorium calibration for different secondary coil positions are valid for stimuli above the threshold as well as for those just at the threshold. In my own work I have used both these methods of varying stimulation strength very freely, and have encountered no conditions which would suggest that they are not perfectly interchangeable.

The applicability of the calibration to various sorts of tissues. Erlanger and Garrey suggest (p. 421) that inasmuch as the "*Nutzzeit* plays an important rôle in determining the physiological value of currents of short duration" and since the *Nutzzeit* varies in different kinds of tissue, a calibration that is valid for one kind of tissue might prove erroneous when applied to another. Here, again, we have a question whose answer must be sought in direct experimentation. I find, upon referring to the series of 104 experiments cited in earlier paragraphs, that in 47 of them frogs' muscles, *uncurarized*, in 8 frogs' muscles, *curarized*, in 47 frogs' sciatic nerves, and in the remaining 2

areas of human skin, were stimulated. The calibration applied as accurately to the experiments with any one of these tissues as to the experiments with any other. This general applicability of the calibration signifies that by means of it the relative strengths of stimuli as applied to any one class of tissues, no matter which one, can be accurately expressed. It does not show whether or not relationships can be stated accurately between stimuli applied to tissues having different *Nutzzeiten*. The chief significance of the *Nutzzeit*, to judge from the observations of Gildemeister,[13] is in situations in which it is exceptionally long. In ordinary laboratory tissues, in which the stimulus, even of a rapid induction shock, definitely outlasts the *Nutzzeit*, it is not probable that the sources of error due to variations in the *Nutzzeit* are so much greater than those mentioned above as inherent in the electrical stimulation of tissues as to require special consideration, in calibration schemes whose ideal is the maximum of serviceability, even at the sacrifice of the last degree of accuracy. In other words, the *Nutzzeit*, as a factor in comparisons of stimuli, is of importance to students of the excitation process as a physiological phenomenon, rather than to users of excitations as means of evoking other physiological activities.

Comparisons of shocks generated by different inductoria. Erlanger and Garrey emphasize the difficulty of making valid quantitative comparisons of shocks generated by different inductoria. In this they are fully justified. As Gildemeister showed,[14] and as I have also pointed out,[15] inductoria of dissimilar construction are likely to produce shocks which have very different physiological effects even though the "contained current" in the shocks compared may be exactly the same. Erlanger and Garrey show (p. 403) that these differences in physiological effect are due to differences in shock contour. I might mention incidentally that in my own comparisons of dissimilar inductoria, calibrated according to the "contained current"

[13] Gildemeister: Zeitschrift fur Biologie, lxii, 1913, 359.
[14] Gildemeister: Archiv fur die gesammte Physiologie, cxxxi, 1910, 604.
[15] Martin: This Journal, xxviii, 1911, 49.

method, I have been able repeatedly by varying the secondary resistance, a procedure which also modifies shock contour, to find a resistance at which the inductoria would give equal physiological effects for equal contained currents. One such case I have described previously.[16]

The remedy for this difficulty is obviously the one suggested by Erlanger and Garrey (p. 448) and earlier by myself,[17] namely, the adoption of a standard of inductorium construction for quantitative work. My continued experience in the calibration of inductoria serves only to emphasize in my own mind the desirability of so doing, although indicating also that a certain degree of latitude may be permitted without completely vitiating the quantitative method. I take this occasion to urge as I have done formerly,[18] the Kronecker specifications as suitable for inductoria which are to be used quantitatively.

A specific objection urged against my calibration scheme by Erlanger and Garrey, applicable only so far as comparisons between different inductoria are concerned, is that the theory upon which I have proceeded demands that the time to maximum of break shocks should be constant, whereas they find, experimentally, that it varies in different inductoria (loc. cit., p. 404). They cite only two experiments in which this variation appears (tables 5 and 6, p. 403). They used different gap intervals in these two experiments, although on the previous page they assert that "the form of a shock is profoundly influenced by certain conditions such as length of the gap. . . . ." Of the three coils they studied, one, coil 1, has relatively such coarse wire in the secondary, and appears, from study of all their data to be so inefficient, as to suggest the desirability of rejecting it as an unsuitable instrument for quantitative work. Their other two coils, numbered 2 and 3, are very dissimilar structurally yet the time to maximum of break shocks generated by them agrees within 7–20 per cent.

[16] Martin: Ibid., 54 (table III).
[17] Martin: Ibid., 53.
[18] Martin: The measurement of induction shocks, 89.

In my opinion, one may safely assume that in well constructed coils, agreeing reasonably with the Kronecker standard, the time to maximum of break shocks will not vary so considerably as to vitiate the calibration. My feeling in this regard is strengthened by the very considerable experience I have had in comparing inductoria, in connection with the preparation of calibrations. I have not encountered such failures of physiological effect to agree with expectation as would occur frequently if the times to maximum of shocks from various coils differed so widely as to vitiate my method.

The knife-blade key. Erlanger and Garrey point out (p. 385) that the mercury key I have described[19] often fails to give clean cut breaks. This defect was pointed out to me also by Professor Dodge of Wesleyan University, who was using the key at the Nutrition Laboratory of the Carnegie Institution of Washington. Forbes, in this laboratory, noted the same defect. Apparently the thin vulcanite blade yielded sidewise somewhat during its rapid movement through the mercury. The defect was completely cured by Dodge by the expedient of inserting, within the mercury chamber in which the blade moves, a strip of watchspring, so adjusted as to press against the blade during its movement. I have substituted in my recent keys a glass vane $\frac{1}{32}$ inch thick, for the vulcanite. This has been tested by Forbes with the string galvanometer and shown to give clean breaks.

SUMMARY

1. Consideration of the conditions of distribution of stimulating currents through tissues leads to the suggestion that an inherent error of unknown amount, assumed to be 10 per cent, limits the degree of accuracy possible of attainment by quantitative stimulation methods, and, therefore, renders unnecessary for practical purposes extreme refinement of such methods.

2. The contention that approximating the coils of an inductorium modifies the contour of the shocks generated by it is

[19] Martin: This Journal, xxvi, 1910, 181.

shown to apply only to the inner portion of the field, within the direct influence of the iron core.

3. Physiological tests demonstrate that when the secondary coil is in the outer part of the field, beyond the direct influence of the iron core, the physiological efficiencies of break shocks generated by it are proportional to their contained currents.

4. Physiological tests demonstrate that a calibration scale for the outer part of the inductorium field, based upon contained currents, is valid when applied to tissues of high resistance as well as when applied to those of low resistance.

5. Physiological tests demonstrate that in the inner portion of the field a calibration that is valid for tissues of low resistance will not hold completely for tissues of high resistance. For practical purposes, however, the calibration is sufficiently accurate to be serviceable as far in as position 6 cm. in a "standard" inductorium.

6. Reference is made to a method, elsewhere described, for taking into account the influence of secondary resistance on the efficiency of induction shocks.

7. The calibration is shown by two distinct lines of evidence to apply to stimuli above the threshold as well as to stimuli at the threshold.

8. The *Nutzzeit* is considered as a possible factor modifying the validity of calibrations. Experimental evidence is offered that a calibration scale which is valid for muscle is equally valid for nerve, although the latter has a shorter *Nutzzeit* than the former. Comparisons of stimuli applied to tissues having different *Nutzzeiten* are admitted to be somewhat uncertain, but the belief is expressed that the error of such comparisons is probably no greater than the other errors inherent in the electrical stimulation of tissues.

9. In order for the shocks generated by different inductoria to be quantitatively comparable, the inductoria should be of reasonably similar construction. The Kronecker specifications are urged as suitable for adoption as standard.

10. A method is described of curing the defect that sometimes appears in the vulcanite knife-blade key. A glass blade is shown to be superior to one of vulcanite.